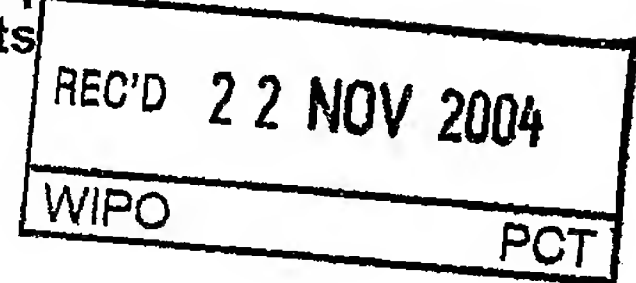




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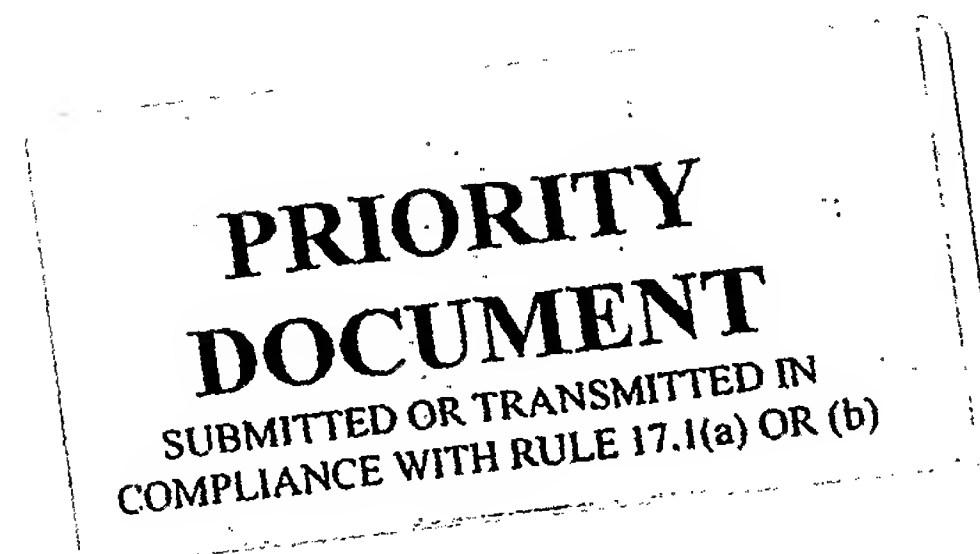
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04004891.0

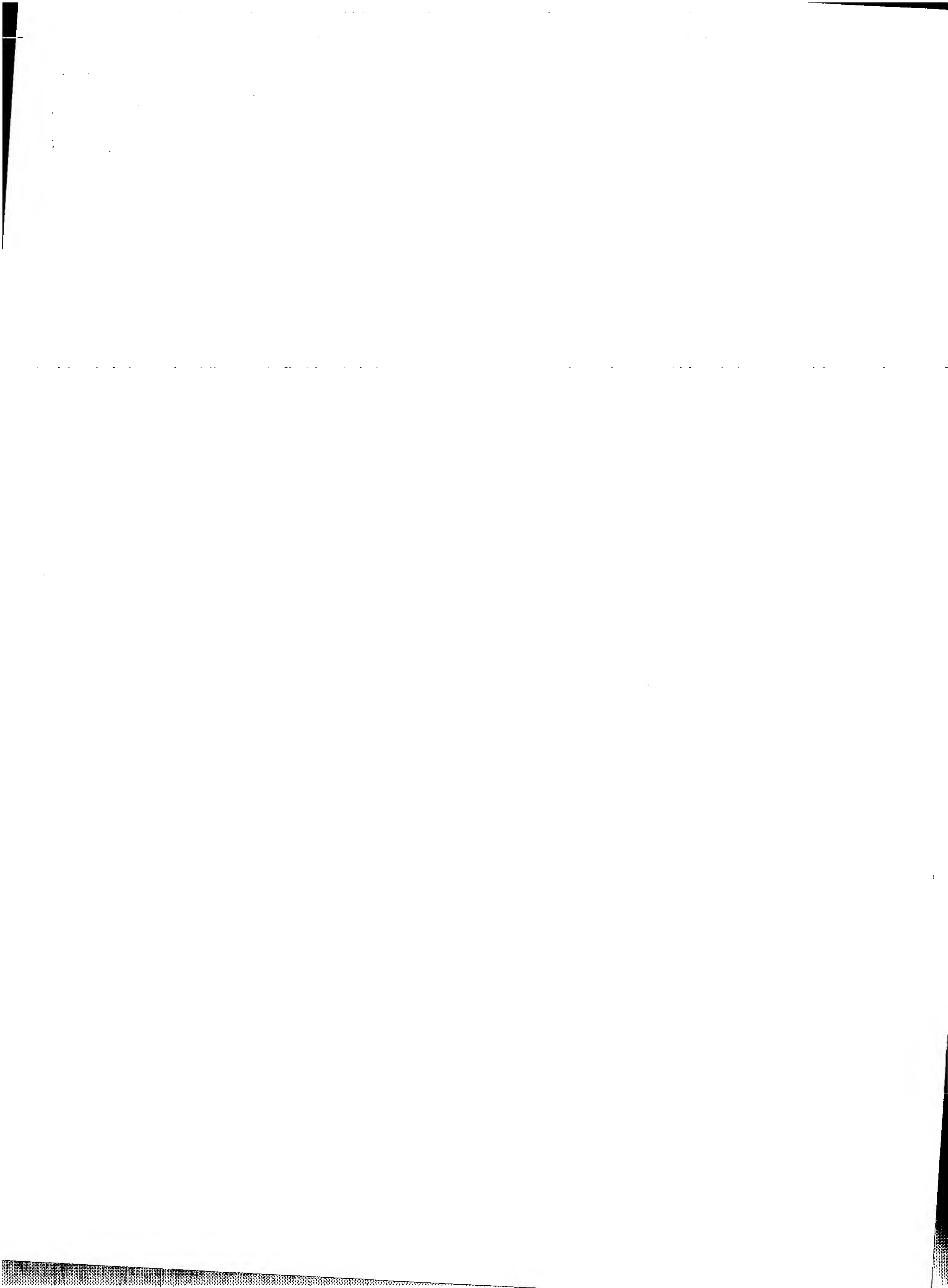


Der Präsident des Europäischen Patentamts
Im Auftrag

For the President of the European Patent O

Le Président de l'Office européen des brev
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R C van Dijk





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Application no.: 04004891.0
Demande no:

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Anmelder/Applicant(s)/Demandeur(s):

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se référer à la description.)

4,7-Dihydro-5H-thieno[2,3c]pyran derivatives and their analogues as effective
compounds against infectious and other diseases

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)
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Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

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PL PT RO SE SI SK TR LI

5 **4,7-Dihydro-5H-thieno[2,3c]pyran derivatives and their analogues as effective
 compounds against infectious and other diseases.**

Description

10 The present invention relates to 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and
their analogues and pharmaceutically acceptable salts thereof, the use of these
derivatives for the prophylaxis and/or treatment of mycobacteria-induced infections,
opportunistic infections, autoimmune diseases, bipolar disorders, cardiovascular
15 diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative
diseases, and stroke as well as compositions containing at least one 4,7-dihydro-5H-
thieno[2,3c]pyran derivative or analogue thereof and/or pharmaceutically acceptable
salts thereof.

20 Mycobacteria is the cause for a number of severe diseases, among them
tuberculosis, leprosy, and mycobacteria-induced meningitis. Tuberculosis is an
ancient scourge of human beings, caused by *Mycobacterium tuberculosis*. Although
more than three billion people have been inoculated with the vaccine BCG, presently
more than 50,000 people die every week of tuberculosis world-wide, and there are
estimations that one third of the world's population is infected by *Mycobacterium*
25 *tuberculosis*. According to a recent report of the World Health Organisation (WHO)
on tuberculosis epidemic, distributed via the internet, it is estimated that between the
years 2000 and 2020, nearly one billion people will carry tuberculosis bacteria, 200
million people will get sick, and 35 million will die of tuberculosis, if control of the
disease and preventive measures are not strengthened. Moreover, it has been
30 reported that 32% of HIV infected individuals die of tuberculosis. The situation has
become even more dramatic since a number of *Mycobacterium tuberculosis* strains
have shown a multidrug resistance, which cannot be attacked by conventional
therapy, e.g. antibiotics. In addition, immune-suppressed people like AIDS patients
are often victims of mycobacterial infections leading to a poor prognosis.

35 There are several reasons why mycobacteria-induced diseases are difficult to cure:
First of all, mycobacteria can perform a differentiation process called "dormancy" or
"persistency". Dormant mycobacteria are much more resistant against conventional
antibacterial drug treatment. Secondly, many of the mycobacteria species have long

replication times, resulting in a slow growth. One consequence thereof is that antimycobacterial drugs need longer medication times compared to the medication of faster growing pathogenic bacteria. Both factors cited above are reasons why a medical treatment of mycobacteria-induced diseases has to last at least for several months. A third factor why conventional antibacterial drug treatment is so difficult with regard to mycobacteria-induced diseases is that these bacteria have a relatively thick cell wall, which is not or almost not permeable for many substances.

The use of 4,5,6,7-tetrahydrobenzo[b]thiophene derivatives in the treatment of mycobacterial infections such as tuberculosis are described in the as yet unpublished PCT patent application PCT/EP03/03697. The compounds described therein have been found to be effective in blocking the activity of mycobacterial protein serine/threonine kinases, particularly protein kinase G (PknG), which have been identified as an essential component involved in the persistence and enhanced survival of pathogenic mycobacterial within a macrophage cell line, and thereby provide a mode for the elimination of mycobacteria.

Additionally, biologically active 4,7-dihydro-5-H-thieno[2,3c]pyran and 4,7-dihydro-5-H-thieno[2,3-c]thiopyran derivatives are described in *Biorg. Med. Chem. Letters* **2002**, *12*, 1897-1900, in which compounds which inhibit TNF- α -production are described, in *J. Med. Chem.* **2002**, *45*, 4443-4459, in which compounds are described which act as protein-tyrosine phosphatase 1B (PTP1B) inhibitors, or in Japanese patent JP 2002308870, in which compounds are described, which act as *Staphylococcus aureus* inhibitors. Further derivatives are described in *Armianskii Khimicheskii Zhurnal* **1987**, *40*(9), 581-7. These references do not disclose any PkNG inhibitory activity for these compounds.

In WO 01/98290 thiophene derivatives are described as active kinase inhibitors.

One important feature for pharmaceutical active agents in general is that these agents have a high degree of metabolic stability. It was found that the compounds described in PCT/EP03/03697, while being pharmaceutically active as PkNG inhibitors, left room for further increase of metabolic stability.

Taking into account the above-mentioned problems with conventional antimycobacterial treatment, it is the object of the present invention to provide compounds and/or pharmaceutically acceptable salts thereof which can be used as pharmaceutically active substances, especially for the prophylaxis and/or treatment of mycobacteria-induced infections, a method to treat mycobacteria-induced

diseases by means of those compounds, as well as compositions comprising at least one of those compounds and/or pharmaceutically acceptable salts thereof as pharmaceutically active ingredients.

- 5 A further object is to provide compounds and/or pharmaceutically acceptable salts thereof which can be used as pharmaceutically active substances for the prophylaxis and/or treatment of autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke.

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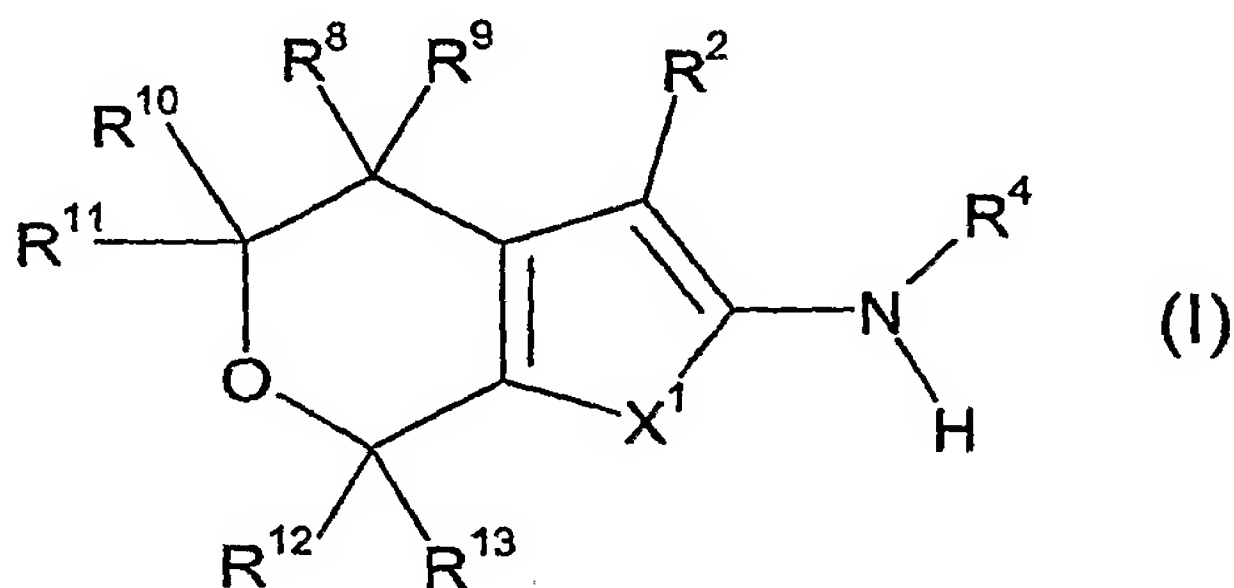
These objects are solved by the 4,7-Dihydro-5H-thieno[2,3c]pyran derivative and analogous compounds and/or their pharmaceutically acceptable salts of independent claim 1, the compound according to claim 25, the use of at least one of the those compounds and/or the pharmaceutically acceptable salts thereof as pharmaceutically active agents according to independent claim 26, the use of the compounds for the preparation of a medicament for the treatment of various diseases according to independent claims 27 and 36, the use of the compounds as an inhibitor for a protein kinase according to independent claim 45, and the use of at least one compound and/or a pharmaceutically active salt thereof for the preparation of a pharmaceutical composition according to independent claim 51. Further advantageous features, aspects and details of the invention are evident from the dependent claims, the description, the examples and the drawings.

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The 4,7-Dihydro-5H-thieno[2,3c]pyran derivatives and analogues thereof according to the present invention are represented by the following general formula (I)

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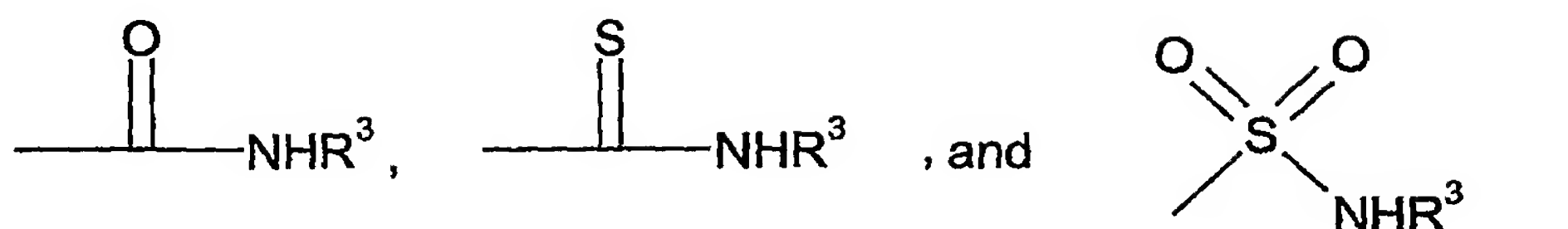
wherein

X¹ is selected from S, O, NR¹,

and R¹ is selected from H, substituted or unsubstituted C₁-C₆-alkyl,

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R^2 is selected from



10

wherein R^3 is selected from H, HO-substituted, H_2N -substituted or HS-substituted $\text{C}_1\text{-C}_6$ -alkyl,

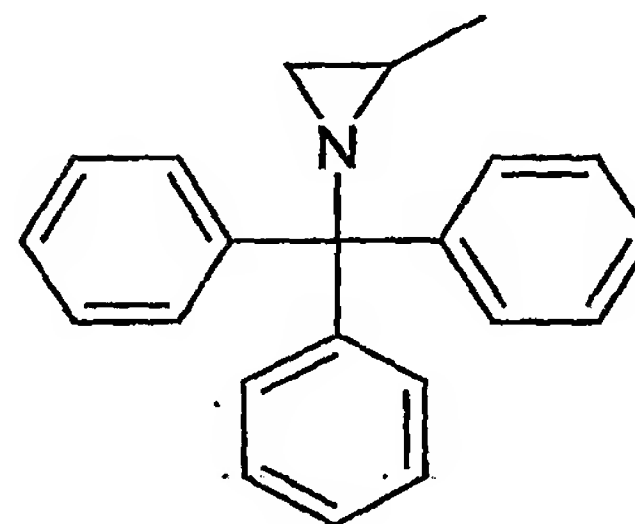
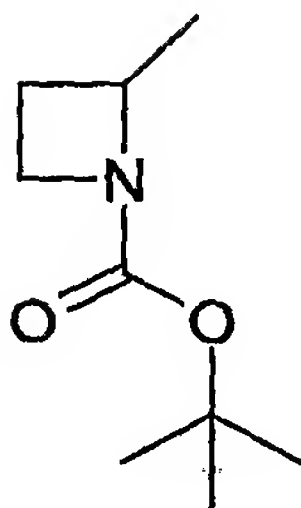
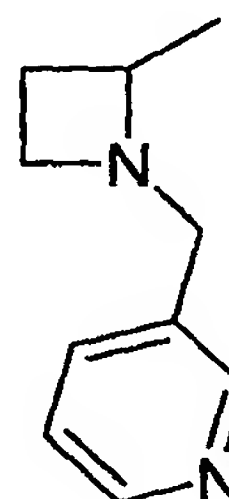
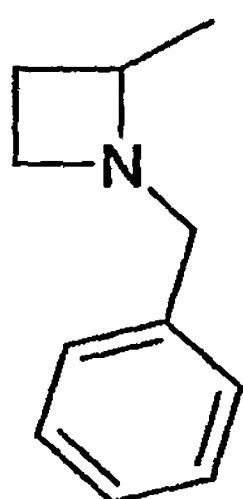
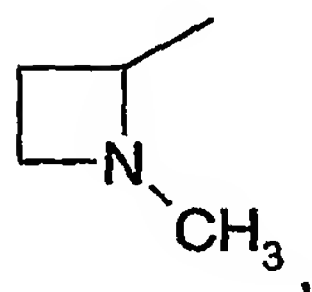
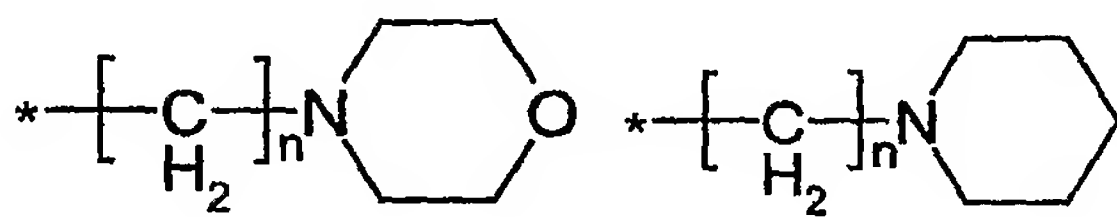
R^4 is selected from H, $-\text{C}(=\text{X}^2)\text{R}^5$ and $-\text{SO}_2\text{R}^5$,

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wherein X^2 is O, S or NH and

R^5 is selected from substituted or unsubstituted $\text{C}_3\text{-C}_6$ -cycloalkyl, $\text{C}_1\text{-C}_6$ -alkyl, aryl, heteroaryl, heterocycloalkyl, $\text{C}_2\text{-C}_4$ -alkenyl, $\text{C}_2\text{-C}_4$ -alkinyl, adamantyl,

20



or $-(\text{CH}_2)_n\text{-NR}_{14}\text{R}_{15}$,

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wherein R_{14} and R_{15} are independently selected from substituted or unsubstituted $\text{C}_1\text{-C}_4$ -alkyl or $\text{C}_2\text{-C}_4$ -alkenyl and wherein $n = 1$ to 6,

or NR^6R^7 ,

wherein

R^6 is selected from H, C_1 - C_6 -alkyl, and

R^7 is selected from substituted or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, aryl, heteroaryl, heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, or adamantyl,

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R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1 - C_6 -alkyl

R^{10} is selected from H, substituted or unsubstituted C_1 - C_6 -alkyl, C_1 - C_6 -alkoxy, or OH

R_{11} is selected from H and substituted or unsubstituted C_1 - C_6 -alkyl

R_{12} is selected from H and substituted or unsubstituted C_1 - C_6 -alkyl, C_1 - C_6 -alkoxy, or

10 OH, and

R^{13} is selected from H or substituted or unsubstituted C_1 - C_6 -alkyl,

and include stereoisomeric and regioisomeric forms and pharmaceutically acceptable salts of these compounds.

15

As used in the present invention, the term substituted or unsubstituted C_1 - C_6 -alkyl or C_1 - C_4 -alkyl or C_1 - C_3 -alkyl is meant to include linear or branched alkyls in which optionally one, two or three of the hydrogen atoms bonded to the carbon chain are substituted by a halogen atom such as F, Cl, Br, or I, preferably F or Cl, a -OH or -

20 SH group, a - NH_2 group, methoxy or ethoxy group, or phenyl group. These terms therefore especially comprise, depending on the number of carbon atoms in each respective term, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, $-C_5H_{11}$, $-CH_2-C(CH_3)_3$, $-CH(CH_3)-C_3H_7$, $-CH_2-CH(CH_3)-C_2H_5$, $-CH(CH_3)-CH(CH_3)_2$, $-C(CH_3)_2-C_2H_5$,25 $-CH_2-C(CH_3)_3$, $-C_2H_4-CH(CH_3)_2$, $-C_6H_{13}$, $-C_3H_6-CH(CH_3)_2$, $-C_2H_4-CH(CH_3)-C_2H_5$, $-CH(CH_3)-C_4H_9$, $-CH_2-CH(CH_3)-C_3H_7$, $-CH(CH_3)-CH_2-CH(CH_3)_2$, $-CH(CH_3)-CH(CH_3)-C_2H_5$, $-CH_2-CH(CH_3)-CH(CH_3)_2$, $-CH_2-C(CH_3)_2-C_2H_5$, $-C(CH_3)_2-C_3H_7$, $-C(CH_3)_2-CH(CH_3)_2$, $-C_2H_4-C(CH_3)_3$, $-CH(CH_3)-C(CH_3)_3$,

30 the above described manner, especially to give phenyl substituted alkyls such as benzyl.

Similarly, the term substituted or unsubstituted C_3 - C_6 -cycloalkyl is meant to include cyclolalkanes in which optionally one, two or three of the hydrogen atoms bonded to the carbon atoms of the cycle are substituted by a halogen atom such as F, Cl, Br, or I, preferably F or Cl, a -OH or -SH group, a - NH_2 , methoxy or ethoxy or methyl, ethyl or phenyl group. This term therefore includes cyclopropyl, cyclobutyl,

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cyclopentyl, cyclohexyl as well as methyl substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, ethyl substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or phenyl substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, optionally substituted in the above described manner.

5

Similarly, the term unsubstituted or substituted C₂-C₄-alkenyl is meant to include branched or linear alkenyles in which optionally one, two, three or four of the hydrogen atoms bonded to the carbon atoms of the alkyl are substituted by a halogen atom such as F, Cl, Br, or I, preferably F or Cl. These terms therefore are meant to comprise ethenyl, *cis*-prop-1-enyl, *trans*-prop-1-enyl, *cis*-prop-2-enyl, *trans*-prop-2-enyl, but-1-enyl, *cis*-but-2-enyl, *trans*-but-2-enyl, but-3-enyl, optionally substituted in the above described manner.

10

Similarly, the term unsubstituted or substituted C₂-C₄-alkinyl is meant to include branched or linear alkynyles in which optionally one, two, three or four of the hydrogen atoms bonded to the carbon atoms of the alkyl are substituted by a halogen atom such as F, Cl, Br, or I, preferably F or Cl. These terms therefore are meant to comprise prop-1-ynyl, prop-2-ynyl, but-1-ynyl, but-2-ynyl, and but-3-ynyl, optionally substituted in the described above manner.

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The term substituted or unsubstituted aryl is meant to include aromatic compounds, in which one, two or three of the hydrogen atoms bonded to the aromatic ring are substituted by an halogen, such as F, Cl, Br or I, preferably F and Cl, or substituted by -NO₂, -OH, -SH, -NH₂, -CN, methyl, acetyl or methoxy. This term is therefore meant to comprise phenyl, 2,3-halogen substituted phenyl, 3,4-halogen substituted phenyl, as well as, for instance, 4-acetylphenyl, 4-methylphenyl or 4-fluorophenyl.

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The term substituted or unsubstituted heteroaryl is meant to include aromatic groups in which the aromatic ring comprises at least one heteroatom selected from the group N, O, or S, and in which one, two or three of the hydrogen atoms bonded to the aromatic ring are optionally substituted by an halogen, such as F, Cl, Br or I, preferably F and Cl, or substituted by -NO₂, -OH, -SH, methyl or methoxy. This term therefore includes furanyl, pyrrolyl, thienyl, and pyridinyl which optionally can be substituted in the above described manner.

35

The term substituted or unsubstituted heterocycloalkyl is meant to include cycloalkyles in which at least one of the carbon atoms of the ring, preferably 1 or 2 atoms, have been substituted by a heteroatom selected from the group consisting of N, O, and S which optionally and in which one, two or three of the hydrogen atoms

bonded to the ring are substituted by an halogen, such as F, Cl, Br or I, preferably F and Cl, or substituted by methyl or methoxy. This term therefore includes pyrrolidinyl; piperidinyl and tetrahydrofuranyl, which optionally can be substituted in the above described manner.

5

In a preferred embodiment of the present invention X^1 is S.

In a further preferred embodiment of the present invention X^1 is NR^1 , and R^1 is selected from H, substituted or unsubstituted C_1 - C_6 -alkyl, and preferably is methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl, or benzyl.

10

In a further preferred embodiment of the present invention X^1 is O.

In a further preferred embodiment of the present invention R^2 is $-C(=O)NHR^3$ and R^3 is selected from H, HO-substituted, H_2N -substituted or HS-substituted C_1 - C_4 -alkyl, and preferably is H.

15

In a further preferred embodiment of the present invention R^2 is $-C(=S)NHR^3$ and R^3 is selected from H, HO-substituted, H_2N -substituted or HS-substituted C_1 - C_4 -alkyl, and preferably is H.

20

In a further preferred embodiment of the present invention R^2 is $-SO_2NHR^3$ and R^3 is selected from H, HO-substituted, H_2N -substituted or HS-substituted C_1 - C_4 -alkyl, and preferably is H.

25

In yet another preferred embodiment of the invention R^3 is selected from the group consisting of H, $-CH_2-CH_2-OH$, $-CH_2-CH_2-NH_2$, $-CH_2-CH_2-SH$, $-CH_2-CH(OH)-CH_3$, $-CH_2-CH(SH)-CH_3$, or $-CH_2-CH(NH_2)-CH_3$.

In a further preferred embodiment of the present invention R^4 is $-C(=X^2)R^5$ and X^2 is O or S, and preferably O.

30

In a further preferred embodiment of the present invention R^4 is $-SO_2-R^5$.

In yet another preferred embodiment of the invention R_5 is selected from the group consisting of substituted or unsubstituted methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1 - C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, prop-1-enyl, prop-2-enyl, but-1-enyl, but-2-enyl, but-3-enyl, prop-1-ynyl, prop-2-ynyl, but-1-ynyl, but-2-ynyl, but-3-ynyl, adamantyl, or NR^6R^7 , wherein R^6 is H and R^7 is selected from substituted or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, aryl, heteroaryl, heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, or adamantyl.

10

In yet another preferred embodiment of the invention R_5 is selected from the group consisting of substituted or unsubstituted methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1 - C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, prop-1-enyl, prop-2-enyl, but-1-enyl, but-2-enyl, but-3-enyl, prop-1-ynyl, prop-2-ynyl, but-1-ynyl, but-2-ynyl, but-3-ynyl, or adamantyl.

15

In yet another preferred embodiment of the present invention R_5 is selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl-substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, methyl-substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, carboxyl substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, furanyl, methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert.-butyl, prop-1-enyl, but-1-enyl, adamantyl, 3,4-difluorophenyl or NR^6R^7 , wherein R^6 is H and R^7 is selected from substituted or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, aryl, heteroaryl, heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, or adamantyl, and R^7 preferably is selected from the group consisting of substituted or unsubstituted C_3 - C_6 -cycloalkyl, preferably cyclohexyl, or an unsubstituted or substituted phenyl. In a further preferred embodiment of the present invention, R^7 is selected from the group consisting of mono-, di- or tri-substituted phenyl groups, wherein the substituents are selected from the group consisting halogen, such as F, Cl, Br or I, preferably F and Cl, or $-NO_2$, $-OH$, $-SH$, $-NH_2$, $-CN$, C_1 - C_6 -alkyl, preferably methyl, acyl, preferably acetyl, or methoxy. In a further embodiment of the present

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invention the group R^7 is selected from the group consisting of phenyl, 3,4-difluorophenyl, 4-acetylphenyl, or 4-methylphenyl.

In another preferred embodiment of the present invention R^7 is selected from substituted or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, heteroaryl, heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, or adamantyl. In a further embodiment of the present invention, the compound 5,5-dimethyl-2-(3-phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide is excluded from the compounds according to the present invention. In another embodiment of the present invention, when R^7 is any one of the groups as outlined above, at least one of the groups R^{10} and R^{11} is not methyl and preferably are one or both of these groups is hydrogen.

In yet another embodiment of the present invention R^7 is selected from substituted or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, aryl, heteroaryl, heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, or adamantyl, and R^{10} is selected from H, substituted or unsubstituted C_1 - C_6 -alkoxy, or OH.

In yet another preferred embodiment of the present invention R^8 is H and R^9 is selected from H, or substituted or unsubstituted C_1 - C_6 -alkyl.

20

In a further preferred embodiment of the present invention R^8 and R^9 are both H.

In a further preferred embodiment of the present invention R^{10} , R^{11} , R^{12} , and R^{13} are independently selected from H and substituted or unsubstituted C_1 - C_6 -alkyl, and preferably from H or methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl or tert.-butyl.

In yet another preferred embodiment of the present invention R^{10} and R^{11} are methyl and R^{12} and R^{13} are H, or R^{10} , R^{11} , R^{12} , and R^{13} are H, or R^{10} , R^{11} , R^{12} , and R^{13} are methyl, or R^{10} and R^{11} are H and R^{12} and R^{13} are methyl.

In yet another preferred embodiment of the present invention R^{10} is selected from substituted or unsubstituted C_1 - C_6 -alkoxy or OH and R^{11} is selected from H or substituted or unsubstituted C_1 - C_6 -alkyl.

30

In yet another preferred embodiment of the present invention R^{12} is selected from substituted or unsubstituted C_1 - C_6 -alkoxy or OH and R^{13} is selected from H or substituted or unsubstituted C_1 - C_6 -alkyl.

- 5 In a further preferred embodiment of the present invention R^1 is selected from the group consisting of methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert.-butyl or benzyl.

10 In a further preferred embodiment of the present invention R_{14} and R_{15} are independently selected from methyl, ethyl and propyl or allyl, and preferably are methyl.

In yet another preferred embodiment of the invention compound according to formula (I) is selected from the group consisting of:

- | | | |
|----|---------------|--|
| 15 | (Compound 1) | 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, |
| | (Compound 2) | 2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, |
| | (Compound 3) | 2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3- |
| 20 | | carboxylic acid amide, |
| | (Compound 4) | 2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, |
| | (Compound 5) | 2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, |
| 25 | (Compound 6) | 2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, |
| | (Compound 7) | 2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, |
| | (Compound 8) | 2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, |
| 30 | | |
| | (Compound 9) | 2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, |
| | (Compound 10) | 2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, |

- (Compound 11) 2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,
- (Compound 12) 2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,
- 5 (Compound 13) 2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,
- (Compound 14) 2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,
- (Compound 15) 2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,
- 10 (Compound 16) 5,5-Dimethyl-2-(3-phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,
- (Compound 17) 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-sulfonamide,
- 15 (Compound 18) 2-(3-Cyclohexyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,
- (Compound 19) 2-(3-Phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,
- (Compound 20) 2-[3-(4-Acetyl-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,
- 20 (Compound 21) 2-(3-p-Tolyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, and
- (Compound 22) 2-[3-(4-Fluoro-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide

25

The present invention also comprises pharmaceutically active salts of these compounds, all stereoisomeric forms and regioisomeric forms of these compounds or prodrugs thereof.

30

Other aspects of the present invention relate to the 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and analogues thereof as outlined above in the general formula (I) for use as new pharmaceutically active agents, particularly for the prophylaxis and/or treatment of virally or bacterially induced diseases or infections, especially infections induced by bacteria of the genus legionella, and especially legionnaires disease, or

35 mycobacteria-induced infections (including opportunistic infections) and diseases, especially mycobacteria induced meningitis, tuberculosis and leprosy,

- pharmaceutical compositions comprising these 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and analogues thereof as active ingredients and a method for treating virally and/or bacterially induced diseases, particularly mycobacteria-induced infections, in mammals, including humans, especially for the treatment of treatment
- 5 of virally or bacterially induced diseases or infections, especially infections induced by bacteria of the genus legionella, and especially legionnaires disease, or mycobacteria-induced infections (including opportunistic infections) and diseases, especially mycobacteria induced meningitis, tuberculosis and leprosy.
- 10 Other diseases which can be successfully treated with the 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and analogues thereof according to the present invention are autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke. What is said above and in the following with regard to the treatment of
- 15 diseases equally applies with respect to the prophylaxis against respective diseases.

Autoimmune diseases, which may be treated with the compounds of the present invention, are e.g. asthma, chronic obstructive pulmonary diseases, systemic lupus erythematosus, immune mediated glomerulonephritis, rheumatoid arthritis,

20 osteoporosis, psoriasis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, alopecia or autoimmune diabetes mellitus.

The same applies not only with respect to the treatment, but also with regard to the prophylaxis against respective diseases.

25 Cardiovascular diseases which may be treated with the compounds of the present invention, are e.g. adult congenital heart disease, aneurysms, angina, angina pectoris, arrhythmias, cardiovascular disease prevention, cardiomyopathies, congestive heart failure, myocardial infarction, pulmonary hypertension, hypertrophic

30 growth, restenosis, stenosis or arteriosclerosis.

A typical cell proliferative disease which can be treated with the compounds of the present invention is cancer, e.g. bladder cancer, breast cancer, cancer of the central nervous system, cancer of the colon, gastric cancer, lung cancer, kidney cancer,

35 melanoma, head and neck cancer, ovarian cancer, cervix cancer, glioblastoma, pancreas cancer, prostate cancer, stomach cancer, skin cancer, testis cancer, leukaemia, Hodgkin's lymphoma, liver cancer and renal cancer.

The diabetes which can be treated with the compounds of the present invention is diabetes Type I and Type II.

5 The inflammation which can be treated with the compounds of the present invention may be mediated by cytokines, such as TNF- α , IL-1 β , GM-CSF, IL-6 and/or IL-8.

10 Among the neurodegenerative diseases which can be treated with the compounds of the present invention are Alzheimer's disease, Parkinson's disease, AIDS-related dementia, Huntington's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, spinal muscular atrophy and cerebellar degeneration.

15 Surprisingly, it was found that the compounds according to the present invention as well as pharmaceutically acceptable salts of these derivatives are effective against virally and/or bacterially induced diseases, especially mycobacteria-induced infections and diseases, as well as autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke at pharmaceutically acceptable concentrations while exhibiting enhanced metabolic stability.

20 Additionally, the present invention relates to the use of the compounds of the present invention for the manufacturing of a pharmaceutical composition for the prophylaxis and/or treatment of virally and/or bacterially induced diseases, particularly those infections and diseases mentioned above, as well as autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, 25 inflammation, neurodegenerative diseases, and stroke.

The compounds of the present invention are effective against mycobacteria induced infections, particularly tuberculosis, but also e.g. leprosy and mycobacteria-induced meningitis. Mycobacteria which induce or cause these infectious diseases are 30 members of the group comprising the tuberculous bacteria *Mycobacterium tuberculosis*, *M. bovis*, *M. africanum* and *M. leprae* as well as the non-tuberculous bacteria *M. abscessus*, *M. avium*, *M. celatum*, *M. chelonae*, *M. fortuitum*, *M. genavense*, *M. gordonae*, *M. haemophilum*, *M. intracellulare*, *M. kansii*, *M. malmoense*, *M. marinum*, *M. scrofulaceum*, *M. simiae*, *M. szulgai*, *M. ulcerans* and 35 *M. xenopi*. Because of the outstanding clinical importance of tuberculosis, microbiologists have distinguished the so-called "Mycobacterium tuberculosis complex" consisting of *Mycobacterium tuberculosis*, *M. bovis*, and *M. africanum* from all other mycobacteria which form the group of the so-called "atypical mycobacteria" or "non-tuberculous mycobacteria (NTM)".

The present invention also provides a method for preventing or treating infections and diseases, especially virally or bacterially induced diseases or infections, more specially infections induced by bacteria of the genus legionella such as legionnaires disease, mycobacteria-induced infections (including opportunistic infections) in mammals (including humans), as well as a method for preventing against and treating diseases, like autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke, which method comprises administering to the mammal an pharmaceutically effective amount of the compounds of the present invention to treat an infection or disease. Especially, the method is used for the treatment of tuberculosis, but also for other mycobacteria-induced infections like leprosy or mycobacteria-induced meningitis.

According to a still further aspect, the present invention refers to pharmaceutical compositions comprising at least one compound according to the present invention as an active ingredient together with at least one pharmaceutically acceptable (i.e. non-toxic) carrier, excipient and/or diluent. The pharmaceutical compositions of the present invention can be prepared in a conventional solid or liquid carrier or diluent and a conventional pharmaceutically-made adjuvant at suitable dosage level in a known way. The preferred preparations are adapted for oral application. These administration forms include, for example, pills, tablets, film tablets, coated tablets, capsules, powders and deposits.

Furthermore, the present invention also includes pharmaceutical preparations for parenteral application, including dermal, intradermal, intragastral, intracutan; intravasal, intravenous, intramuscular, intraperitoneal, intranasal, intravaginal, intrabuccal, percutan, rectal, subcutaneous, sublingual, topical, or transdermal application, which preparations in addition to typical vehicles and/or diluents contain at least one compound according to the present invention and/or a pharmaceutical acceptable salt thereof as active ingredient.

The pharmaceutical compositions according to the present invention containing at least one compound according to the present invention, i.e. one 4,7-Dihydro-5H-thieno[2,3c]pyran derivative or analogues compound thereof as set out in general formula (I) in independent claim 1 or claims dependent thereon, and/or a pharmaceutical acceptable salt thereof as active ingredient will typically be administered together with suitable carrier materials selected with respect to the intended form of administration, i.e. for oral administration in the form of tablets,

capsules (either solid filled, semi-solid filled or liquid filled), powders for constitution, gels, elixirs, dispersable granules, syrups, suspensions, and the like, and consistent with conventional pharmaceutical practices. For example, for oral administration in the form of tablets or capsules, the active drug component may be combined with
5 any oral non-toxic pharmaceutically acceptable carrier, preferably with an inert carrier like lactose, starch, sucrose, cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, talc, mannitol, ethyl alcohol (liquid filled capsules) and the like. Moreover, suitable binders, lubricants, disintegrating agents and coloring agents may also be incorporated into the tablet or capsule. Powders and tablets may contain
10 about 5 to about 95 weight % of the 4,7-dihydro-5H-thieno[2,3c]pyran derivative or analogues compound thereof or the respective pharmaceutically active salt as active ingredient.

Suitable binders include starch, gelatin, natural sugars, corn sweeteners, natural and
15 synthetic gums such as acacia, sodium alginate, carboxymethylcellulose, polyethylene glycol and waxes. Among suitable lubricants there may be mentioned boric acid, sodium benzoate, sodium acetate, sodium chloride, and the like. Suitable disintegrants include starch, methylcellulose, guar gum, and the like. Sweetening and flavoring agents as well as preservatives may also be included, where
20 appropriate. The disintegrants, diluents, lubricants, binders etc. are discussed in more detail below.

Moreover, the pharmaceutical compositions of the present invention may be formulated in sustained release form to provide the rate controlled release of any one
25 or more of the components or active ingredients to optimise the therapeutic effect(s), e.g. antihistaminic activity and the like. Suitable dosage forms for sustained release include tablets having layers of varying disintegration rates or controlled release polymeric matrices impregnated with the active components and shaped in tablet form or capsules containing such impregnated or encapsulated porous polymeric
30 matrices.

Liquid form preparations include solutions, suspensions, and emulsions. As an
example, there may be mentioned water or water/propylene glycol solutions for parenteral injections or addition of sweeteners and opacifiers for oral solutions,
35 suspensions, and emulsions. Liquid form preparations may also include solutions for intranasal administration.

Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be present in combination with a pharmaceutically acceptable carrier such as an inert, compressed gas, e.g. nitrogen.

- 5 For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides like cocoa butter is melted first, and the active ingredient is then dispersed homogeneously therein e.g. by stirring. The molten, homogeneous mixture is then poured into conveniently sized moulds, allowed to cool, and thereby solidified.

- 10 Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions, and emulsions.

- 15 The compounds according to the present invention may also be delivered transdermally. The transdermal compositions may have the form of a cream, a lotion, an aerosol and/or an emulsion and may be included in a transdermal patch of the matrix or reservoir type as is known in the art for this purpose.

- 20 The term capsule as recited herein refers to a specific container or enclosure made e.g. of methyl cellulose, polyvinyl alcohols, or denatured gelatins or starch for holding or containing compositions comprising the active ingredient(s). Capsules with hard shells are typically made of blended of relatively high gel strength gelatins from bones or pork skin. The capsule itself may contain small amounts of dyes, opaquing agents, plasticisers and/or preservatives.

- 25 Under tablet a compressed or moulded solid dosage form is understood which comprises the active ingredients with suitable diluents. The tablet may be prepared by compression of mixtures or granulations obtained by wet granulation, dry granulation, or by compaction well known to a person of ordinary skill in the art.

- 30 Oral gels refer to the active ingredients dispersed or solubilised in a hydrophilic semi-solid matrix.

- 35 Powders for constitution refers to powder blends containing the active ingredients and suitable diluents which can be suspended e.g. in water or in juice.

Suitable diluents are substances that usually make up the major portion of the composition or dosage form. Suitable diluents include sugars such as lactose, sucrose, mannitol, and sorbitol, starches derived from wheat, corn rice, and potato,

and celluloses such as microcrystalline cellulose. The amount of diluent in the composition can range from about 5 to about 95 % by weight of the total composition, preferably from about 25 to about 75 weight %, and more preferably from about 30 to about 60 weight %.

5

The term disintegrants refers to materials added to the composition to support break apart (disintegrate) and release the pharmaceutically active ingredients of a medicament. Suitable disintegrants include starches, "cold water soluble" modified starches such as sodium carboxymethyl starch, natural and synthetic gums such as locust bean, karaya, guar, tragacanth and agar, cellulose derivatives such as methylcellulose and sodium carboxymethylcellulose, microcrystalline celluloses, and cross-linked microcrystalline celluloses such as sodium croscarmellose, alginates such as alginic acid and sodium alginate, clays such as bentonites, and effervescent mixtures. The amount of disintegrant in the composition may range from about 2 to about 20 weight % of the composition, more preferably from about 5 to about 10 weight %.

Binders are substances which bind or "glue" together powder particles and make them cohesive by forming granules, thus serving as the "adhesive" in the formulation. Binders add cohesive strength already available in the diluent or bulking agent. Suitable binders include sugars such as sucrose, starches derived from wheat corn rice and potato, natural gums such as acacia, gelatin and tragacanth, derivatives of seaweed such as alginic acid, sodium alginate and ammonium calcium alginate, cellulose materials such as methylcellulose, sodium carboxymethylcellulose and hydroxypropylmethylcellulose, polyvinylpyrrolidone, and inorganic compounds such as magnesium aluminum silicate. The amount of binder in the composition may range from about 2 to about 20 weight % of the composition, preferably from about 3 to about 10 weight %, and more preferably from about 3 to about 6 weight %.

Lubricants refer to a class of substances which are added to the dosage form to enable the tablet granules etc. after being compressed to release from the mould or die by reducing friction or wear. Suitable lubricants include metallic stearates such as magnesium stearate, calcium stearate, or potassium stearate, stearic acid, high melting point waxes, and other water soluble lubricants such as sodium chloride, sodium benzoate, sodium acetate, sodium oleate, polyethylene glycols and D,L-leucine. Lubricants are usually added at the very last step before compression, since they must be present at the surface of the granules. The amount of lubricant in the composition may range from about 0.2 to about 5 weight % of the composition,

preferably from about 0.5 to about 2 weight %, and more preferably from about 0.3 to about 1.5 weight % of the composition.

5 Glidents are materials that prevent caking of the components of the pharmaceutical composition and improve the flow characteristics of granulate so that flow is smooth and uniform. Suitable glidents include silicon dioxide and talc. The amount of glident in the composition may range from about 0.1 to about 5 weight % of the final composition, preferably from about 0.5 to about 2 weight %.

10 Coloring agents are excipients that provide coloration to the composition or the dosage form. Such excipients can include food grade dyes adsorbed onto a suitable adsorbent such as clay or aluminum oxide. The amount of the coloring agent may vary from about 0.1 to about 5 weight % of the composition, preferably from about 0.1 to about 1 weight %.

15

To identify substances for drug development against mycobacteria-induced diseases, it was searched for inhibitors of signal transduction components present in mycobacteria. As already mentioned above, the elimination of mycobacteria from the human body is presently achieved by inhibiting the growth of respective bacteria
20 by means of antibiotics. According to the present invention, a novel strategy has been used to fight against mycobacteria, namely to attack mycobacterial signal transduction components which are involved in the persistence of the bacteria within the host cell. Previously, it had been shown that mycobacteria penetrate cells via the endocytotic pathway. Endosomes containing non-pathogenic mycobacteria fuse
25 to lysosomes and subsequently the bacteria are degraded by lysosomal enzymes. However, pathogenic mycobacteria, like *Mycobacterium tuberculosis*, contain additional "virulence genes" which prevent fusion of endosomes and lysosomes and thus circumvent the degradation within a host cell.

30 Mycobacterial protein serine/threonine kinases, particularly protein kinase G (PknG), have been identified as an essential component involved in the persistence and enhanced survival of pathogenic mycobacteria within a macrophage cell line. Furthermore, it could be demonstrated that the activity of PknG is an essential factor for virulence of mycobacteria. In accordance with the present invention, compounds
35 have been found which are blocking the activity of PknG in a submicromolar range thus showing that PknG is a suitable target for recognising diseases, monitoring diseases, and controlling therapy of diseases related to mycobacterial infections. These compounds (inhibitors) were able to induce efficient degradation of

mycobacteria within host cells so that the present invention provides a novel mode for elimination of mycobacteria.

5 With the compounds according to the present invention, besides protein kinases, the activity of further proteins and enzymes, respectively, can be influenced. Such further proteins and enzymes are e.g. nucleotide binding proteins, ATP-binding proteins,, and kinases, such as lipid kinases. The currently known protein kinases which can be affected with the compounds of the present invention are shown in Table III at the end of the specification.

10

It has been found that certain disease inducing factors can be secreted by a cellular organism to the environment of the organism. Specifically, in the present case it has been found that mycobacterial proteins are secreted from the bacterium *Mycobacterium tuberculosis* to the environment of such a bacterium. A protein, which
15 can be secreted by *Mycobacterium tuberculosis* is the protein serine/threonine kinase PknG. The fact that the above-mentioned inventive compounds are particularly effective against PknG may be due to the fact that this protein kinase can be attacked by these compounds without the need to penetrate the (thick) cell wall of *Mycobacterium tuberculosis*.

20

The compounds according to the present invention are obtainable by different synthetic routes. One route, which leads to 4,7-dihydro-5H-thieno[2,3c]pyran derivatives starts with the reaction of tetrahydro-pyran-4-one or a correspondingly substituted derivative thereof with an cyano-acetate ester under acidic or basic
25 conditions, preferably under acidic conditions, and under elimination of water and subsequent reaction of the reaction product with sulfur in the presence of an organic base to give a corresponding 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester derivative.

30 As a next step, the amino group in the thus obtained 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester derivative can be acylated to give a corresponding 2-carboxylamino compound. As an acylation reagent a carboxylic acid chloride is preferably used. This reaction can optionally be carried out in the presence of a base such as an tertiary amide, preferably NEt(ⁱPr)₂.

35

Other suitable reactions to obtain the secondary carboxylic acid amides can be used, for instance reaction of the amino group with a carboxylic acid and a coupling-agent as used in peptide chemistry, such as HOBt, HOObt, HBTU or HOAt.

Alternatively, if instead of the acyl group a sulfonyl group is to be attached to the amino group in 2-position, the 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester derivative can be reacted with a sulfonyl chloride compound to give a corresponding 2-sulfanylamino derivative.

5

The thus obtained compounds can then optionally be reacted with bromine in the presence of an organic acid, preferably acetic acid, to substitute one hydrogen in 7-position of the heterocyclic nucleus by a hydroxyl group.

- 10 The above described 3-carboxylic acid ester derivative compounds can then be reacted in a subsequent reaction step with an alkali metal amide, such as LiNH_2 or NaNH_2 , in a polar solvent, which is essentially inert to the alkali metal amide, to give the corresponding 3-carboxylic acid amide derivative. This reaction is preferably carried out under the exclusion of moisture and optionally under an inert atmosphere.
- 15 The application of lithium amide instead of sodium amide results in higher yields and purer products.

- To prepare the corresponding 4,7-dihydro-5H-thieno[2,3-c]pyran derivatives in which a sulfonamide is attached in 3-position, in a first step, 4,7-dihydro-5H-thieno[2,3-c]pyran-2-amine can be acylated, preferably using a carboxylic acid chloride to give the corresponding 2-carbonyl-amino derivative. This compound can then be reacted with sulfonylchloride, preferably under an inert atmosphere and subsequently with ammonia to give the 3-sulfonamide compound.
- 20

- 25 If the compounds used to synthesise the compounds according to the present invention contain -NH, -SH or -OH functional groups which potentially interfere with the desired reaction, these may of course be protected with suitable protective groups, which can later on be removed from the respective compounds.

- 30 To obtain those analogues of the 4,7-dihydro-5H-thieno[2,3c]pyran derivatives in which the S-atom in the 5-membered ring of the heterocyclic nucleus is substituted either by NR^1 or O, the following synthetic approach can be utilized, which is partially based on a method described in Hauser, C.R., Hoffenberg, D.S.; *J.Org.Chem.* 1955, 20, 1448 - 1453.

35

To obtain the O-analogue compounds the amino group in 2-position a corresponding 2-Amino-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane derivatives can be acylated in a first reaction step, using the acylation reaction described above with reference to the acylation of the 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester

derivatives, i.e. preferably using a carboxylic acid chloride as an acylation agent, optionally in the presence of a tertiary amine base such as $\text{NEt}(\text{iPr})_2$.

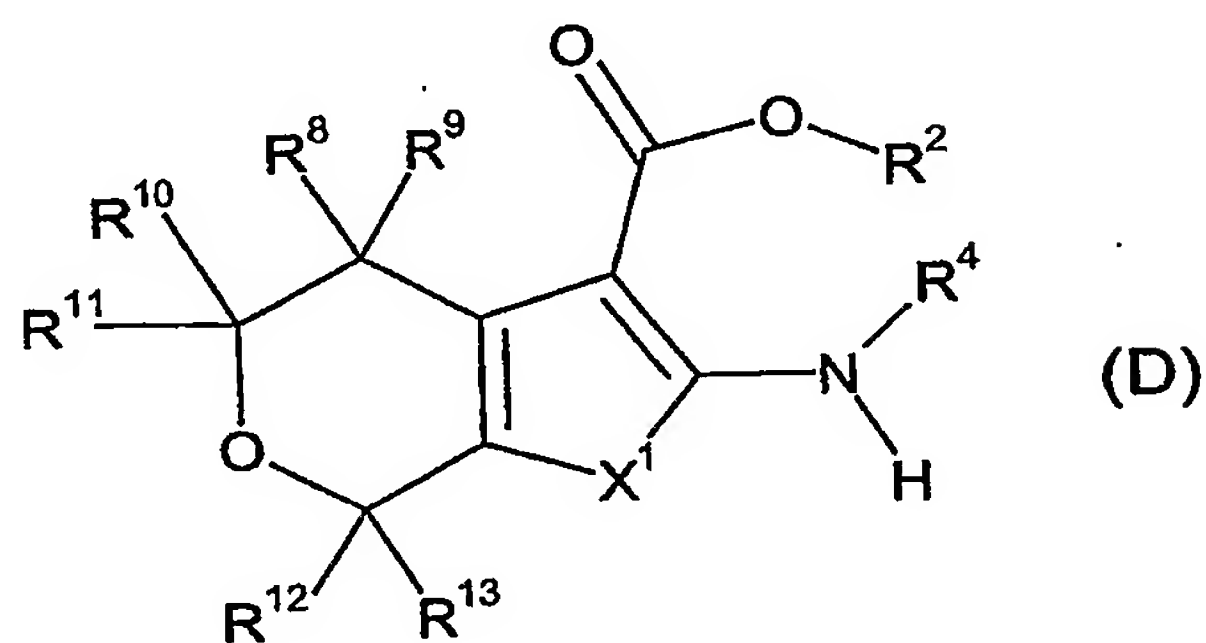
5 Similarly, to obtain the NR^1 -analogue compounds, a corresponding 2-Amino-3-Cyano-4,7-dihydro-5H-pyrrolo[2,3-c]pyrane derivative is acylated in the above described manner.

10 The respective 2-carbonyl-amino derivatives obtained by this acylation can then be reacted with boron trifluoride-acetic acid complex $[\text{BF}_3 \cdot (\text{HOAc})_2]$ and subsequently treated with an aqueous alkali metal hydroxide solution, such as sodium hydroxide, to convert the cyano group in 3-position of the heterocyclic nucleus into the carboxamide group.

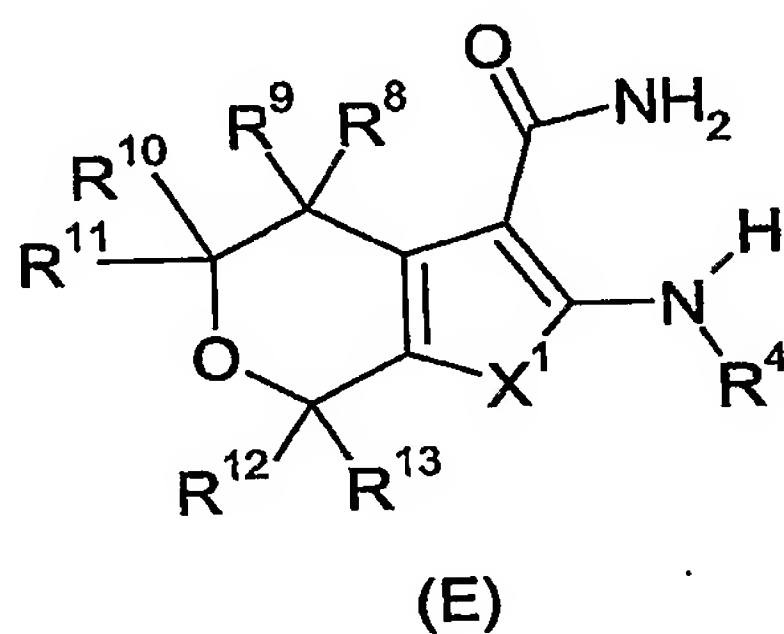
15 In a further aspect of the present invention, the invention is directed at a method for amidation of an carboxylic acid ester to give the corresponding primary carboxylic acid amide. This amidation comprises the step of reacting an carboxylic acid ester with an alkali metal amide in the presence of a polar solvent, which is essentially inert against the alkali metal amide. Preferably, the molar ratio of carboxylic acid ester to alkali metal amide lies in the range of 1:1 to 1: 15., more preferably in the range of
20 1:5 to 1:13 and most preferably in the range of 1:9 to 1: 13.

25 In a preferred embodiment of the method of the present invention, the alkali metal amide is LiNH_2 or NaNH_2 , and preferably is LiNH_2 . The solvent is preferably absolute ether or absolute tetrahydrofuran, preferably tetrahydrofuran, and the reaction is preferably carried out under the exclusion of moisture. Preferably, the reaction is carried out at a temperature of 15°C to 35°C , preferably at 25°C . It is furthermore preferred that the reaction duration lies in the range of from 40 to 80 hours, preferably from 45 to 75 hours.

30 In a preferred embodiment of the method of the present invention, the carboxylic acid ester is a compound according to the following general formula (D):



which is amidated to give the primary carboxylic acid amide according to formula (E),



wherein in formulas (D) and (E)

- 20 X^1 is selected from S, O, or NR^1 , and R^1 is selected from H, substituted or unsubstituted C_1 - C_6 -alkyl,

R^2 is linear or branched C_1 - C_6 alkyl or aryl and preferably is methyl, ethyl, phenyl or benzyl,

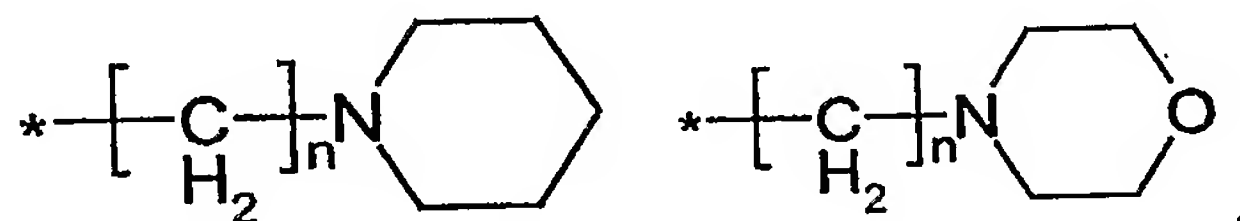
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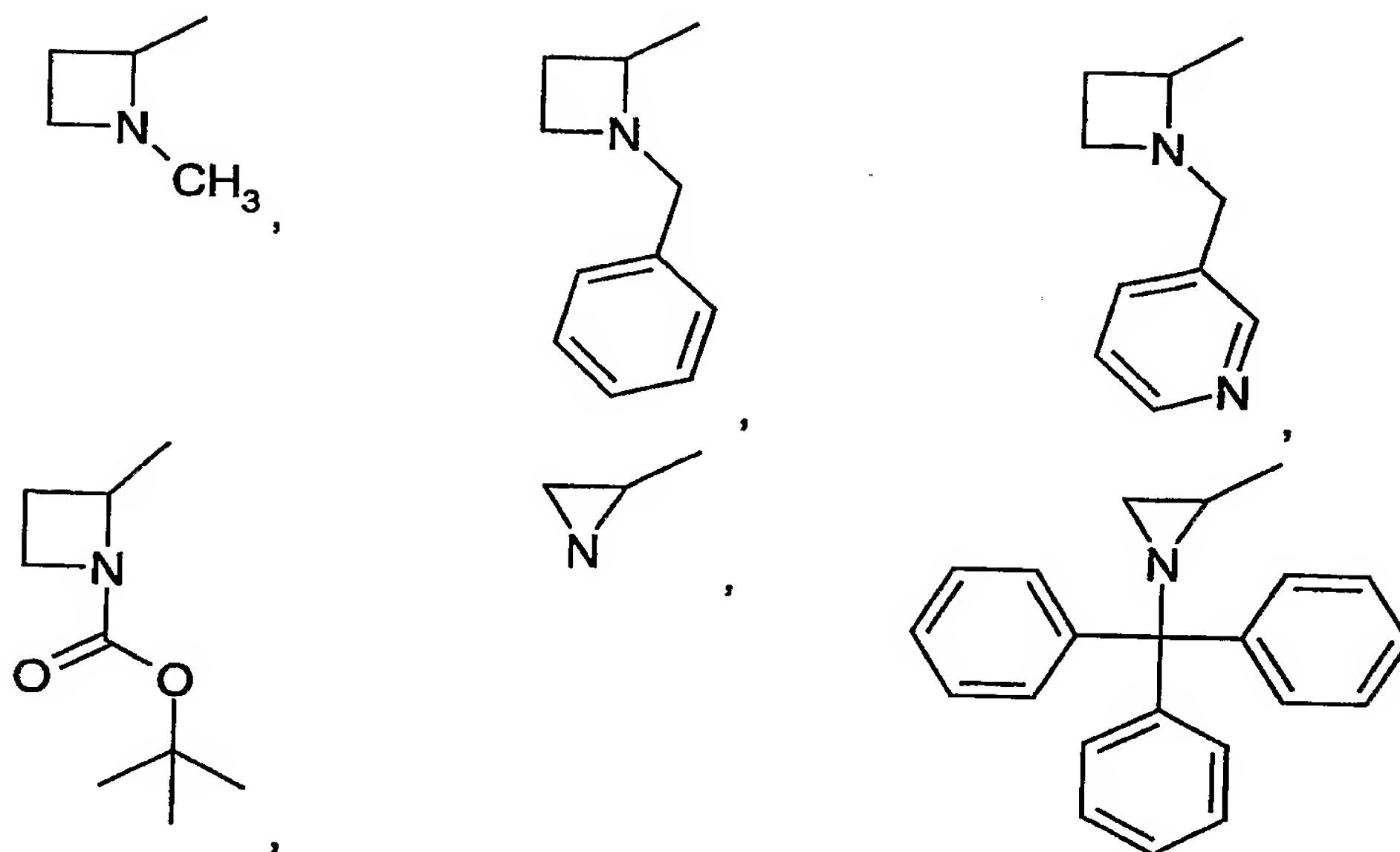
R^4 is selected from H, $-C(=X^2)R^5$ and $-SO_2R^5$,

wherein X^2 is O, S or NH and

R^5 is selected from substituted or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, aryl, heteroaryl, heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, adamantyl,

30





or $-(CH_2)_n-NR_{14}R_{15}$,

wherein R_{14} and R_{15} are independently selected from substituted or unsubstituted C_1 - C_4 -alkyl or C_2 - C_4 -alkenyl and wherein $n = 1$ to 6,

or NR^6R^7 ,

wherein

R^6 is selected from H, C_1 - C_6 -alkyl, and

R^7 is selected from substituted or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, aryl, heteroaryl, heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, or adamantyl,

R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1 - C_6 -alkyl

R^{10} is selected from H, substituted or unsubstituted C_1 - C_6 -alkyl, C_1 - C_6 -alkoxy, or OH

R_{11} is selected from H and substituted or unsubstituted C_1 - C_6 -alkyl

R_{12} is selected from H and substituted or unsubstituted C_1 - C_6 -alkyl, C_1 - C_6 -alkoxy, or OH, and

R^{13} is selected from H or substituted or unsubstituted C_1 - C_6 -alkyl,

and stereoisomeric and regioisomeric forms and pharmaceutically acceptable salts of these compounds.

In a further preferred embodiment of the method of the present invention, in general formulas (D) and (E)

X^1 is S

R^2 is methyl or ethyl,

R^4 is $-C(=O)R^5$ and R^5 is selected from methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1 - C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, *cis*-prop-1-enyl, *trans*-prop-1-enyl, *cis*-prop-2-enyl, *trans*-prop-2-enyl, but-1-enyl, *cis*-but-2-enyl, *trans*-but-2-enyl, but-3-enyl, prop-1-ynyl, prop-2-ynyl, but-1-ynyl, but-2-ynyl, but-3-ynyl or adamantyl,

R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1 - C_6 -alkyl,

R^{10} is selected from H, substituted or unsubstituted C_1 - C_6 -alkyl, C_1 - C_6 -alkoxy, or OH,

R_{11} is selected from H and substituted or unsubstituted C_1 - C_6 -alkyl,

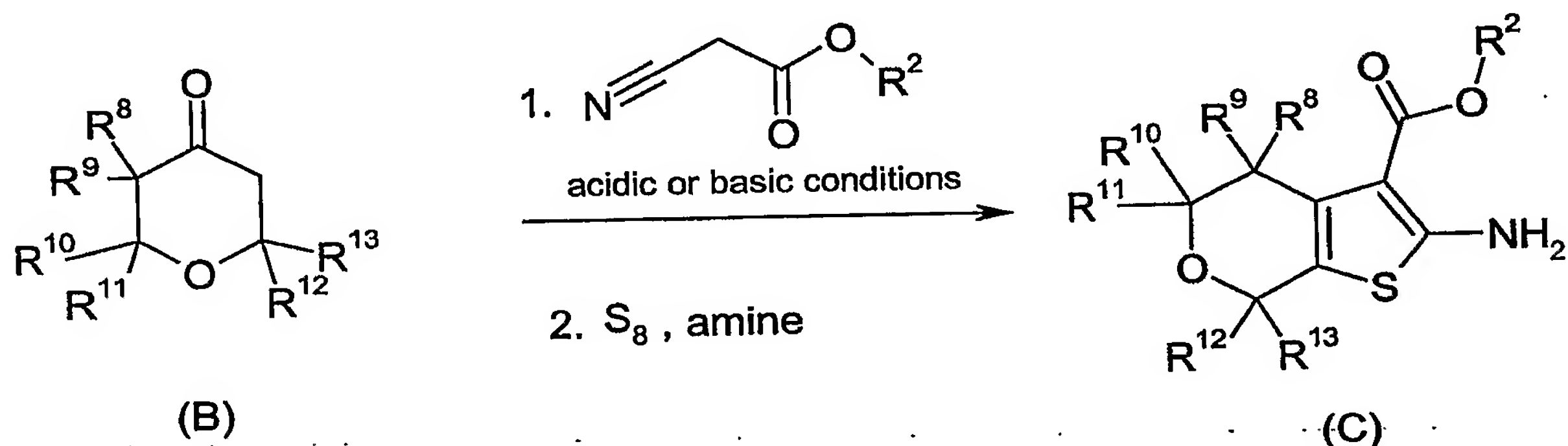
R_{12} is selected from H and substituted or unsubstituted C_1 - C_6 -alkyl, C_1 - C_6 -alkoxy, or OH, and

R^{13} is selected from H or substituted or unsubstituted C_1 - C_6 -alkyl.

15

According to one preferred embodiment of the method of the present invention, the compound according to the general formula (E) is obtained by the following reaction sequence:

20 Step I:

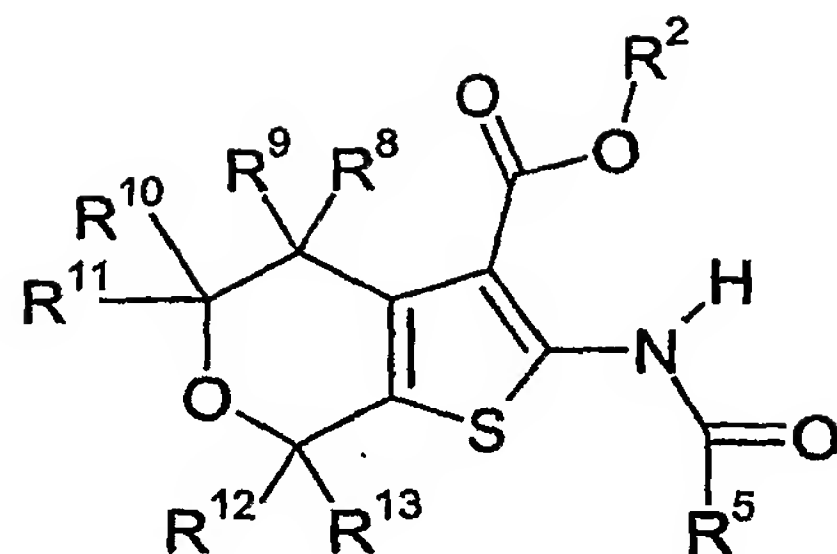


Step II: acylation of the $-NH_2$ group in 2-position in compound C with $R^5C(=O)LG$, wherein LG represents a suitable leaving group, preferably a halogen such as F, Cl, Br or I, most preferably Cl, to give compound (D):

40

5

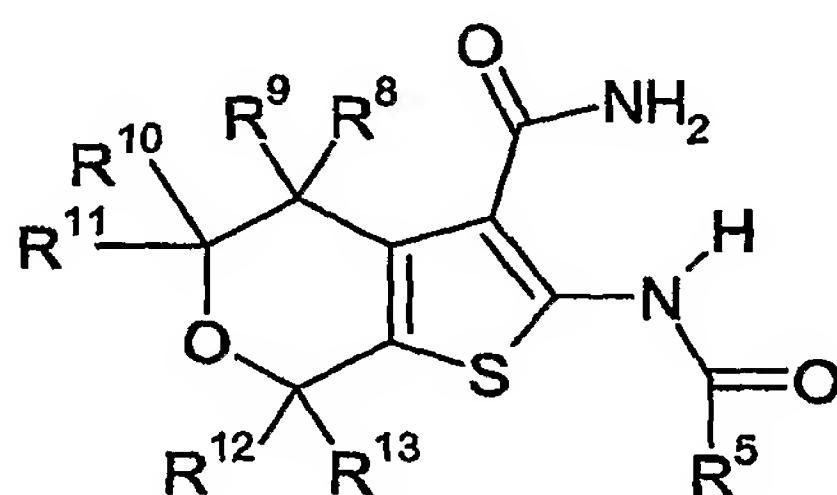
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(D)

and,

- 20 Step III: Amidation of as outlined in any one of claims 60 to 65,
to give compound (E):



(E)

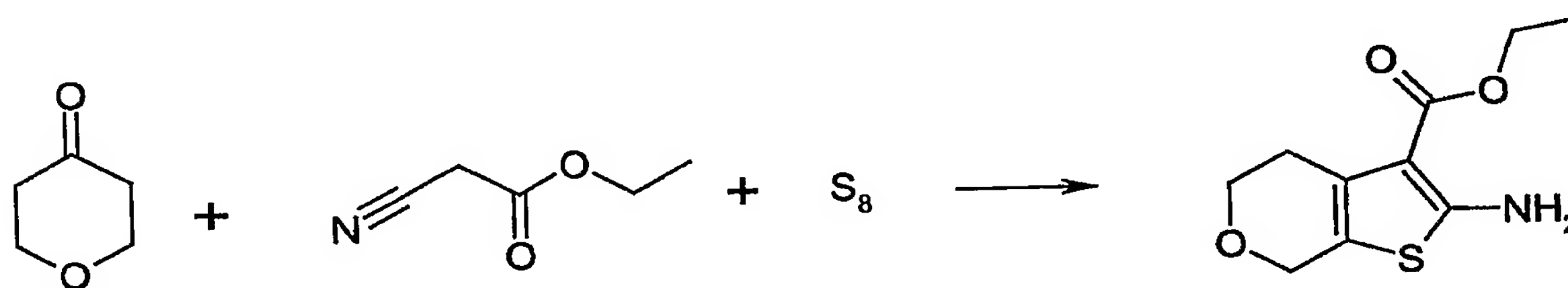
- It is preferred that in Step I the reaction of compound (B) with the cyano-acetate ester is carried out in a nonpolar solvent, preferably benzene, with the addition of a mixture of ammonium acetate and acetic acid in a molar ratio of greater than 1, preferably in the range from 0.5:1 to 0.8 to 1, and preferably at a temperature in the range of 50 to 100 °C, preferably between 70 to 90 °C, preferably under removal of water formed in the reaction, and preferably for a duration of 2 to 4 hours.
- 25 Furthermore, in a preferred embodiment of the present invention, in Step I the reaction product of the reaction of compound (B) with the cyano-acetate ester is reacted with the S₈ in a protic solvent, preferably EtOH, S₈ being added at least in equimolar quantities, preferably in an excess of up to 1.5, more preferably of up to 1.2, in the presence of a amine base, preferably morpholine, at a reaction temperature of between 25 to 65 °C, preferably between 40 and 60 °C, and preferably for a duration of 2 to 6 hours.
- 30
- 35

Examples

- Chemicals were purchased from Sigma-Aldrich. Waters alliance LC system, equipped with Micromass Quadrupole MS detector was used for the purity analysis. NMR data were measured with a Bruker 300 MHz NMR spectrometer.

Syntheses of compounds

10 I. Preparation of Ethyl 2-amino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylate

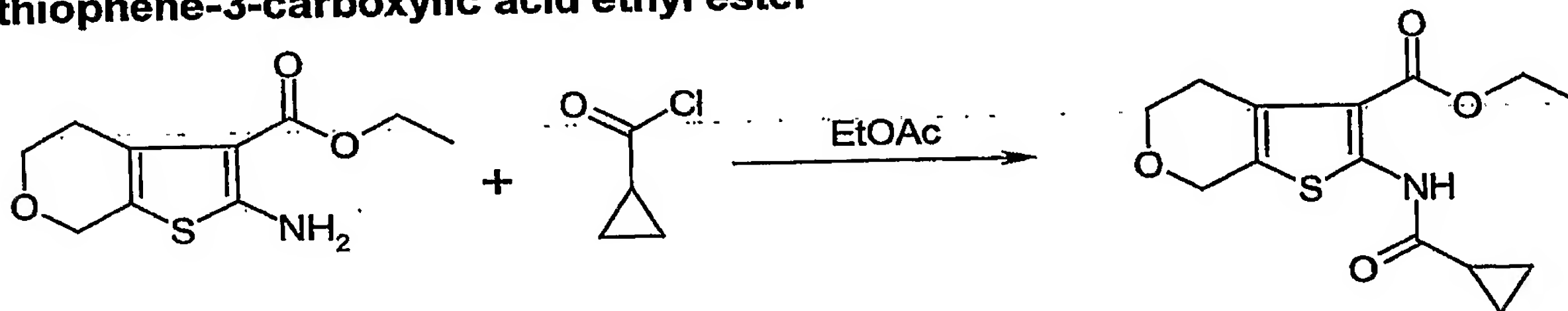


- 1 mM tetrahydro-pyran-4-one, 106 μ L (1 mM) ethyl-cyano-acetate, 0.15 mM ammonium-acetate, and 0.2 mM acetic acid were dissolved in 3 mL benzene and stirred at reflux temperature in a round-bottomed flask equipped with water-remover trap, for 3 hours. The reaction mixture was washed with 2 mL 10% K₂CO₃ solution, dried, and evaporated to dryness. The solid material was dissolved in 1.5 mL EtOH and was stirred with 1.05 mM sulphur and 0.575 mM morpholine at 45-50 °C, for 4 hours. The reaction mixture was evaporated to dryness, washed with n-hexane and isopropylalcohol. This reaction step was developed starting from a procedure described by Gewald, K; Schinke, E; Böttcher, H; *Chem. Ber.* **1966**, 99, 974.

Yield: 57 %

NMR: 7.28 (s, 2H), 4.43 (s, 2H), 4.16 (q, 2H), 3.79 (t, 2H), 3.67 (t, 2H), 1.25 (t, 3H)

25 II. Preparation of 2-(Cyclopropanecarbonyl-amino)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester



- 1 mM cyclopropanecarbonyl chloride was added dropwise to a well stirred, 15 mL ethylacetate solution of 301 mg (1.00 mM) 2-amino-6-phenyl-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester. The reaction mixture was stirred for

3 hours, then diluted to 50 mL, washed two times with water, dried with MgSO_4 , and evaporated to dryness. The product was washed with n-hexane and isopropanol.
Yield: 42 %

NMR: 11.19 (s, 1H), 4.60 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.78 (t, 2H), 2.03 (m, 1H),
5 1.33 (t, 3H), 0.93 (m, 4H)

Analogous to this method the following compounds were also synthesized:

- 10 **2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester** (Yield 57%), NMR: 11.90 (s, 1H), 8.06 (s, 1H), 7.39 (d, 1H), 6.79 (dd, 1H), 4.66 (s, 2H), 4.35 (q, 2H), 3.86 (t, 2H), 2.82 (t, 2H), 1.35 (t, 3H);
- 2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester** (Yield 67%), NMR: 11.36 (s, 1H), 4.62 (s, 2H), 4.32 (q, 2H), 3.84 (t, 2H), 2.79 (t, 2H), 2.06 (bs, 2H), 1.90 (s, 8H), 1.72 (s, 6H), 1.33 (t, 3H);
- 15 **2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester** (Yield 70%), NMR: 11.10 (s, 1H), 4.61 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.78 (t, 2H), 1.90 (d, 2H), 1.69 (m, 3H), 1.43-1.18- (m, 9H);
- 2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester** (Yield 57%), NMR: 11.15 (s, 1H), 4.60 (s, 2H), 4.25 (q, 2H), 3.64 (t, 2H), 2.78 (t, 2H), 1.80 (m, 1H), 1.33 (t, 3H), 1.11 (d, 3H), 0.79 (m, 1H);
- 20 **2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester** (Yield 76%), NMR: 10.91 (s, 1H), 4.61 (s, 2H), 4.28 (q, 2H), 3.83 (t, 2H), 3.44 (m, 1H), 2.78 (bs, 2H), 2.23 (m, 4H), 1.97 (m, 1H), 1.83 (m, 1H), 1.31 (t, 3H);
- 25 **2-Acetylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester** (Yield 85%), NMR: 10.93 (s, 1H), 4.61 (s, 2H), 4.29 (q, 2H), 3.84 (t, 2H), 2.77 (t, 2H), 2.24 (s, 3H), 1.32 (t, 3H);
- 2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester** (Yield 64%), NMR: 10.93 (s, 1H), 4.61 (s, 2H), 4.29 (q, 2H), 3.84 (t, 2H), 2.77 (t, 2H), 2.24 (s, 3H), 1.32 (t, 3H);
- 30 **2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester** (Yield 76%), NMR: 11.02 (s, 1H), 6.90 (m, 1H), 6.35 (dd, 1H), 4.63 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.79 (t, 2H), 1.92 (s, 3H), 1.89 (s, 3H), 1.32 (t, 3H);
- 2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester** (Yield 69%), 11.05(s,1H),4.62(s,2H),4.30(q,2H), 3.84(t,2H), 2.80(t,2H), 2.59(m,1H), 1.64(m,1H), 1.50(m,1H), 1.32(t,3H), 1.14(d,3H), 0.87(t,3H);
- 35 **2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester** (yield 76%), NMR: 11.05(s,1H),4.62(s,2H),4.30(q,2H),

3.84(t,2H), 2.80(t,2H), 2.59(m,1H), 1.64(m,1H), 1.50(m,1H), 1.32(t,3H), 1.14(d,3H), 0.87(t,3H);

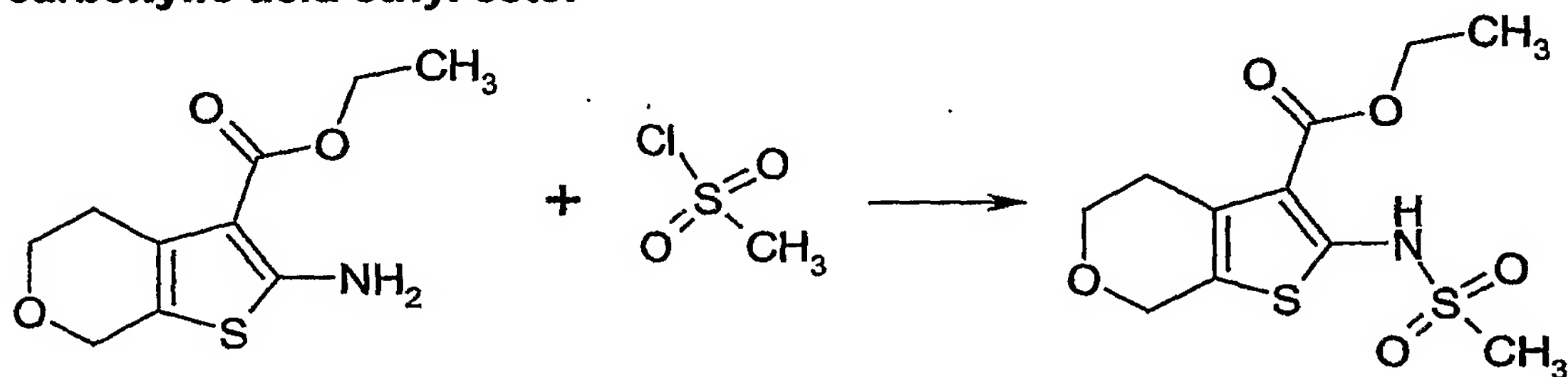
2-(2-Chloro-acetylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 82%), NMR: 11.64 (s, 1H), 4.64 (s, 2H), 4.61 (s, 2H), 4.32 (q, 2H), 3.85 (t, 2H), 2.80 (t, 2H), 1.32 (t, 3H);

2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 79%), NMR: 11.89 (s, 1H), 7.95 (m, 1H), 7.76 (m, 2H), 4.67 (s, 2H), 4.34 (q, 2H), 3.87 (t, 2H), 2.82 (t, 2H), 1.34 (t, 3H);

2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 80%), NMR: 11.08 (s, 1H), 4.62 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.78 (m, 3H), 1.32 (t, 3H), 1.17 (d, 6H);

2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 77%), NMR: 11.04 (s, 1H), 4.61 (s, 2H), 4.29 (q, 2H), 3.84 (t, 2H), 2.99 (m, 1H), 2.78 (t, 2H), 1.91 (m, 2H), 1.65 (m, 6H), 1.31 (t, 3H).

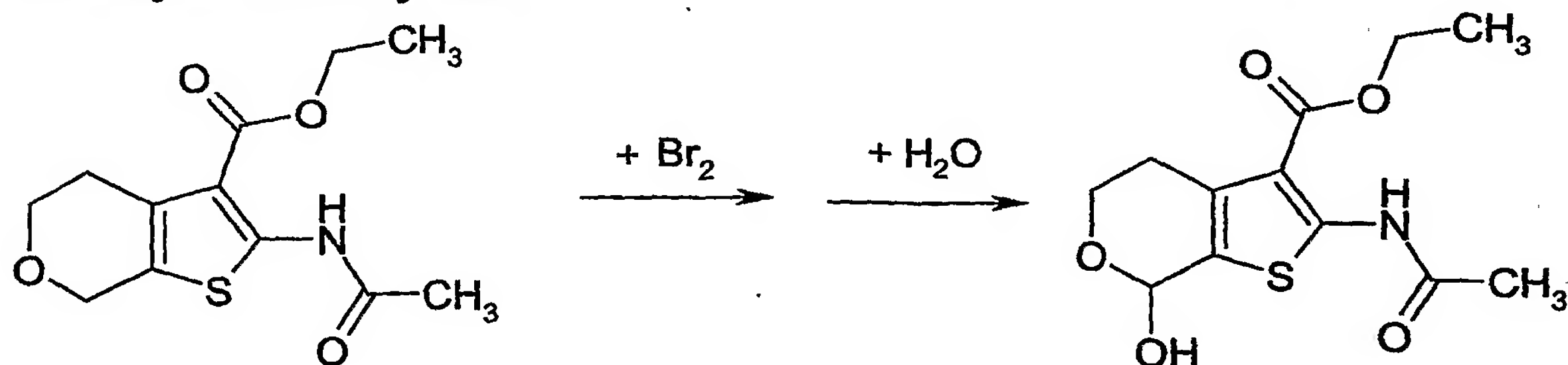
III. Preparation of 2-Methanesulfonylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester



1 mmol 2-Amino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was dissolved in 10 ml benzene and 348 μ L (2.5 equivalent) triethylamine, 195 μ L (2.5 equiv.) methanesulfonyl chloride was added. The reaction mixture was refluxed for 8 hours. The mixture was extracted with 1x 15mL water, 1x 15 mL NaHCO₃, then 1x 15 ml water, 1x 15 mL 1N HCl and saturated NaCl solution. The organic layer was dried above MgSO₄, the solvent was evaporated to vacuo and the residue was crystallized from hexane-isopropanol. (TLC-Eluent: Hexan-Ethylacetate: 2:1)

Yield: 65%, NMR: 11.03 (s, 1H), 4.73 (s, 2H), 4.28 (q, 2H), 3.89 (t, 2H), 3.53 (s, 3H), 2.83 (t, 2H), 1.29 (t, 3H).

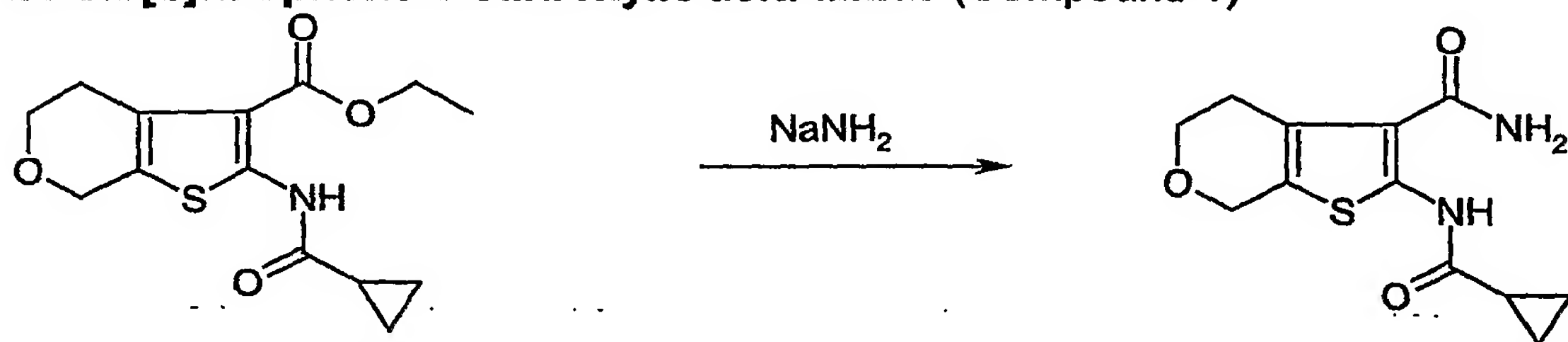
IV. Preparation of 2-Acetamido-7-hydroxy-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester



269 mg (1 mmol) 2-Acetyl-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was dissolved in 15 mL acetic acid and 82 mg (1 mmol) sodium-acetate was added to the mixture, then heated to 55 °C. 159 mg bromine in 15 mL acetic acid was added slowly to the mixture. After one hour stirring it was evaporated under reduced pressure and extracted three times with ethyl acetate and 15 mL water. The organic layer was washed with 10 mL NaHCO_3 solution and dried with MgSO_4 . The solution was evaporated under reduced pressure and the product was crystallized from hexane. The product was washed with IPA, and recrystallized with diisopropyl-ether. Yield: 39% NMR: 10.92 (s, 1H), 4.83 (d, 1H), 4.73 (d, 1H), 4.49 (d, 1H), 4.29 (q, 2H), 3.90 (d, 1H), 3.65 (d, 1H), 2.24 (s, 3H), 1.32 (t, 3H).

The compound 2-(Cyclopropanecarbonyl-amino)-7-hydroxy-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was synthesized in a analogous reaction. Yield: 62%, NMR: 11.20 (s, 1H), 5.65 (s, 1H), 4.93-4.65 (m, 2H), 4.34 (q, 2H), 4.24-4.03 (m, 3H), 2.10 (m, 1H), 1.38 (t, 3H), 0.93 (m, 4H).

V. Preparation of 2-(Cyclopropanecarbonyl-amino)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid amide (Compound 1)



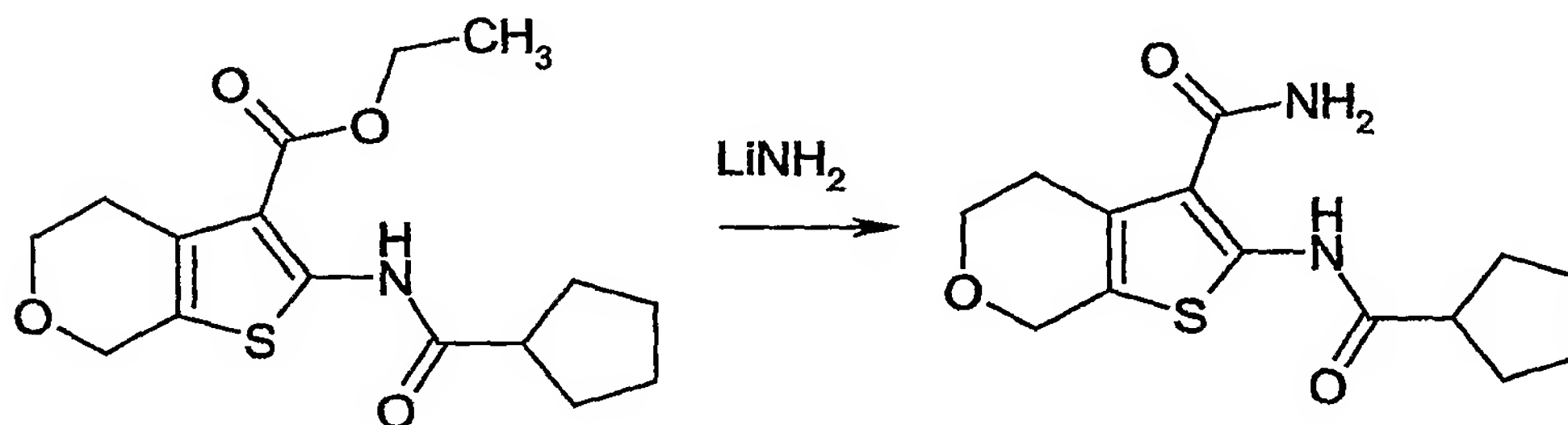
470 mg (12.00 mM) sodium amide was added to the solution of 293 mg (1.00 mM) 2-(cyclopropanecarbonyl-amino)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester in 8 mL abs. tetrahydrofuran. The air-tightly closed reaction mixture was stirred at room temperature for 72 hours.

After the starting material disappeared, the pH of the reaction mixture was set to 5 – 6 with ice cold, 1 N HCl, the precipitated product was filtered off, washed twice with 5 mL n-hexane and dried.

Yield: 89 % white, or off-white crystals; NMR: 11.75 (s, 1H), 4.62 (s, 2H), 3.83 (t, 2H),
5 2.79 (t, 2H), 1.89 (m, 1H), 0.87 (m, 4H)

VI. Preparation of **2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 2)**

10



15

1 mmol 2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was dissolved in 3 mL abs. THF, then 230 mg (10 equivalent) LiNH₂ was added and the mixture was stirred in a stoppered flask at r.t. for 48 hours. The reaction mixture was poured on ice water, the pH of the solution was adjusted to 5 with 5% HCl. The precipitated crystals were filtered out and washed with cold isopropanol. (TLC Eluent: chloroform-MeOH 10:1)

20

Yield: 79%, NMR: 11.73 (s, 1H), 7.2 (bd, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 2.88 (m, 1H),
2.80 (t, 2H), 1.89 (m, 2H), 1.64 (m, 6H).

The following compounds were also prepared by this method:

25

2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 3), Yield: 67%, NMR: 11.77 (s, 1H), 7.2 (bs, 2H), 4.63 (s, 2H),
3.83 (t, 2H), 2.80 (t, 2H), 2.46 (m, 1H), 1.60 (m, 1H), 1.47 (m, 1H), 1.12 (d, 3H), 0.85 (t, 3H);

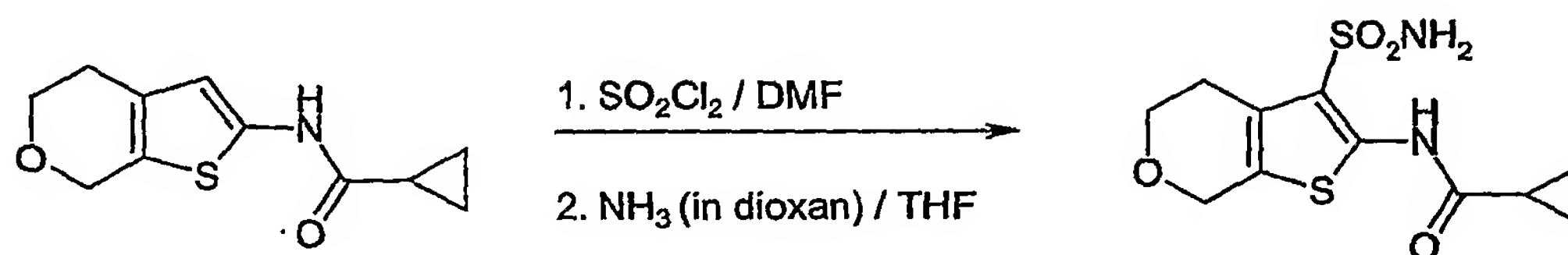
30

2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 4), Yield: 74%, NMR: 11.63 (s, 1H), 7.2 (bs, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 3.33 (m, 1H), 2.81 (t, 2H), 2.20 (m, 4H), 1.97 (m, 1H), 1.83 (m, 1H);
2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 5), Yield: 73%, NMR: 11.26 (s, 1H), 7.32-7.19 (m, 5H), 4.62 (s, 2H), 4.28 (q, 2H), 3.84 (t, 2H), 2.78 (t, 2H), 1.55 (m, 1H), 1.43 (m, 1H), 1.30 (t, 3H);

- 2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 6), Yield: 49%, NMR: 11.64 (s, 1H), 7.3 (bd, 2H), 6.83 (m, 1H), 6.23 (dd, 1H), 4.64 (s, 2H), 3.83 (t, 2H), 2.80 (t, 2H), 1.89 (d, 3H);
- 5 **2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 7), Yield 31%, NMR: 11.56 (s, 1H), 7.2 (bs, 2H), 5.93 (s, 1H), 4.64 (s, 2H), 3.83 (t, 2H), 2.80 (t, 2H), 2.16 (s, 3H), 1.90 (s, 3H);
- 2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 8), Yield 32%, NMR: 12.33 (s, 1H), 7.2 (bs, 2H), 4.64 (s, 2H), 3.83 (t, 2H), 2.83 (t, 2H), 1.22 (s, 9H);
- 10 **2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 9), Yield: 70%; NMR: 13.01 (s, 1H), 7.88 (m, 1H), 7.70 (m, 2H), 7.30 (bs, 2H), 4.69 (s, 2H), 3.86 (t, 2H), 2.86 (t, 2H);
- 2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 10), Yield: 61%, NMR: 11.81 (s, 1H), 7.2 (bd, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 2.81 (t, 2H), 2.67 (m, 1H), 1.14 (d, 6H);
- 15 **2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 11), Yield: 65%, NMR: 11.20 (s, 1H), 5.65 (s, 1H), 4.93-4.65 (m, 2H), 4.34 (q, 2H), 4.24-4.03 (m, 3H), 2.10 (m, 1H), 1.38 (t, 3H), 0.93 (m, 4H);
- 20 **2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 12), Yield: 53%, NMR: 11.71 (s, 1H), 7.5 (bs, 1H), 7.0 (bs, 1H), 4.61 (s, 2H), 3.82 (t, 2H), 2.79 (t, 2H), 1.64 (m, 1H), 1.09 (d, 3H), 0.73 (m, 1H);
- 2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 13), Yield: 34%, NMR: 12.72 (s, 1H), 8.02 (d, 1H), 7.7 (bs, 1H), 7.31 (d, 1H), 7.2 (bs, 1H), 6.76 (dd, 1H), 4.67 (s, 2H), 3.85 (t, 2H), 2.86 (t, 2H);
- 25 **2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 14), Yield: 61%, NMR: 12.23 (s, 1H), 7.3 (b, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 2.83 (t, 2H), 2.03 (s, 3H), 1.86 (s, 5H), 1.70 (s, 5H);
- 30 **2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 15), Yield: 63%, NMR: 11.79 (s, 1H), 7.2 (bd, 2H), 4.63 (s, 2H), 3.82 (t, 2H), 2.80 (t, 2H), 2.41 (m, 1H), 1.89-1.62 (m, 5H), 1.40-1.17 (m, 5H).

Preparation of **2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-sulfonamide** (Compound 17)

35



Sulfurylchloride (13 mmol) was added dropwise to DMF (13 mmol) at 0 °C under Argon. The mixture was stirred for 30 min at 0 °C and 2-(Cyclopropanecarbonylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran (10 mmol) in 2 ml DCM added. The mixture was stirred for 1 h at r.t., diluted with 2 ml of THF and treated with an excess of NH₃ (2 M solution in dioxane, 10 ml, 20 mmol). The mixture was stirred at room temperature overnight. Evaporation of the solvent and recrystallization afforded the title compound.

10 **Preparation of 2-(3-phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 19)**

0.20 g (1.00 mmol) 2-Amino-4,7-dihydro-5H-thieno[2,3-c]thiopyran-3-carboxylic acid amide and 0.06 g (0.50 mmol) 4-dimethylamino-pyridine was dissolved in 10 cm³ absolute 1,4-dioxane. The reaction mixture was treated with 0.14 g, 0.13 cm³ (1.20 mmol) phenylisocyanate in one portion, at room temperature. After stirring for 24 hours at room temperature the solvent was evaporated under reduced pressure. The residue was stirred in the mixture of 15 cm³ water and 5 cm³ ethyl acetate for half an hour, at 0 °C. The product was filtered off, washed with 5 cm³ cold ethyl acetate, and air-dried.

Yield: 0.26 g (82 %)

Mp.: 200-202 °C

Rt: 3.22 min; Mol. Mass: 317

¹H NMR DMSO-d₆ 300MHz, δ(ppm): 10.88 (s, 1H), 10.05 (s, 1H), 7.47 (d, J=8.01 Hz, 2H), 7.35 (broad s, 1H), 7.28 (t, J=7.68 Hz, 2H), 6.99 (t, J=7.32 Hz, 1H), 6.90 (broad s, 1H), 4.62 (m, 2H), 3.83 (m, 2H), 2.80 (m, 2H).

Method II

0.20 g (1.00 mmol) 2-Amino-4,7-dihydro-5H-thieno[2,3-c]thiopyran-3-carboxylic acid amide was dissolved in the mixture of 1 cm³ absolute pyridine and 1 cm³ anhydrous DMSO. The mixture was treated with (1 mmol) isocyanate at room temperature. After stirring for 1 hour at room temperature the reaction mixture was treated with cold 1 N aqueous hydrochloric solution and the precipitate was filtered off, then partitioned between 1 N aqueous hydrochloric solution and ethyl acetate. The organic

phase was collected, dried and concentrated under reduced pressure. The residue was treated with the mixture of ethyl acetate-hexane and the product was obtained by filtration of the crystalline product.

- 5 According to this method the following compounds were prepared:

2-(3-Cyclohexyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 18)

Yield: (32 %)

10 Mp.: 195.7-196.6 °C

Rt: 1.8 min; Mol. Mass: 323

¹H NMR DMSO-d₆ 300MHz, δ(ppm): 10.22(s, 1H), 10.10(s, 1H), 7.3, 7.0 (b, 2H), 4.62(s, 2H), 3.92(m, 1H), 3.82(t, 2H), 2.78(t, 2H), 2.0-1.42(m, 10H)

15 **2-[3-(4-Acetyl-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 20)**

Yield: (81 %)

Mp.: 268-269 °C

Rt: 3.10 min; Mol. Mass: 359

20 ¹H NMR DMSO-d₆ 300MHz, δ(ppm): 11.00(s, 1H), 10.44(s, 1H), 7.89(d, 2H), 7.59(d, 2H), 7.4, 6.9(bs, 2H), 4.61(s, 2H), 3.82(t, 2H), 2.78 (t, 2H), 2.48(s, 3H)

2-[3-(4-Methoxy-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 21)

25 Yield: (81 %)

Mp.: decomposed at 200 °C

Rt: 3.12 min; Mol. Mass: 347

¹H NMR DMSO-d₆ 300MHz, δ(ppm): 10.82(s, 1H), 9.81(s, 1H), 7.36(d, 2H), 6.84(d, 2H), 7.00(b, 2H), 4.59(s, 2H), 3.81(t, 2H), 3.70(s, 3H), 2.77(t, 2H)

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2-[3-(4-Fluoro-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 22)

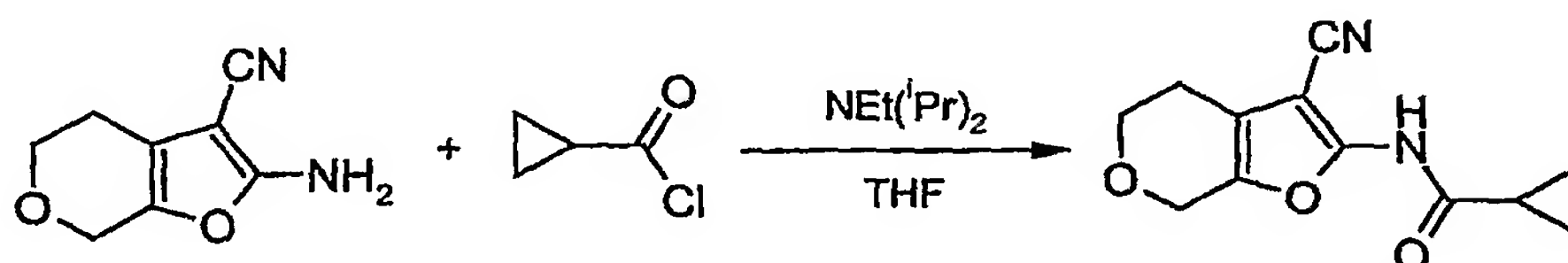
Yield: (80.6 %)

Mp.: decomposed at 280 °C

35 Rt: 3.34 min; Mol. Mass: 335

¹H NMR DMSO-d₆ 300MHz, δ(ppm): 10.91(s, 1H), 10.08(s, 1H), 7.48(m, 2H), 7.45, 6.95(b, 2H), 7.12(t, 2H), 4.61(s, 2H), 3.82 (t, 2H), 2.79(t, 2H)

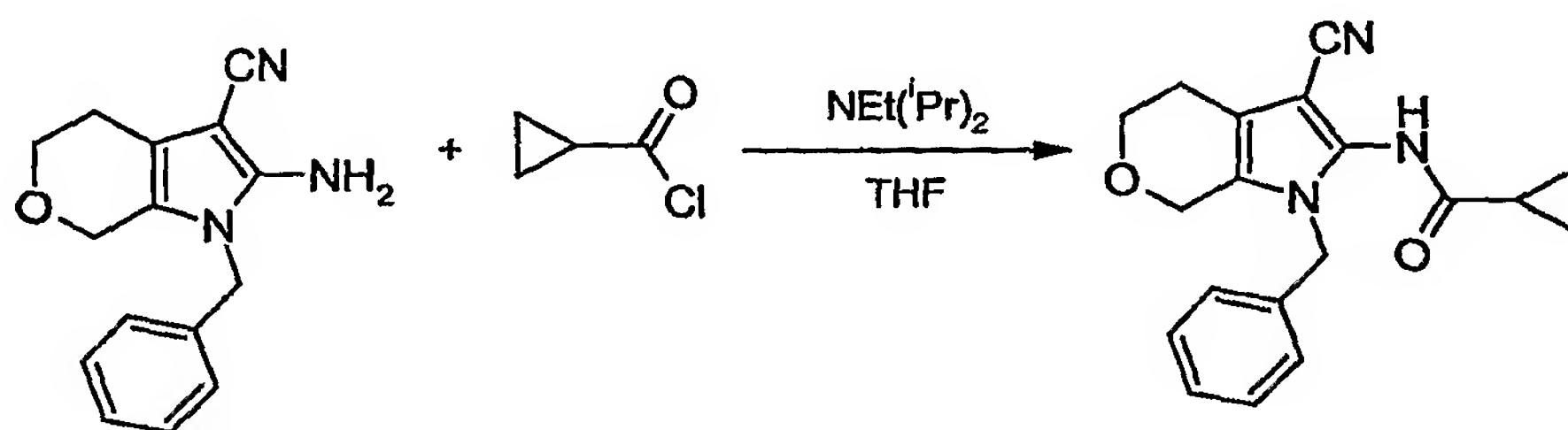
Preparation of 2-[(Cyclopropanecarbonyl)-amino]-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane



- 5 2-Amino-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane (0.66 mmol) and cyclopropylcarbonyl chloride (0.8 mmol) were dissolved in 10 mL of abs. THF. Diisopropylethylamine (0.8 mmol) was added via syringe, and the mixture was stirred overnight at room temperature. After dilution with 20 mL of water, the aqueous phase was extracted four times with ethylacetate, the organic layer washed once with water, 10 dried over sodium sulfate and the solvents evaporated. Recrystallization of the crude material from hot ethanol gave the desired product.

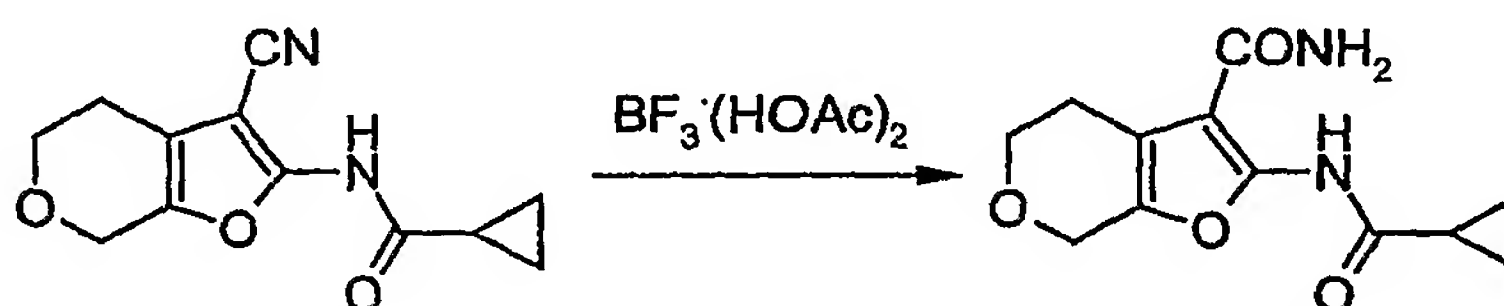
Preparation of 1-Benzyl-2-[(Cyclopropanecarbonyl)-amino]-3-Cyano-4,7-dihydro-5H-pyrrolo[2,3-c]pyrane

15



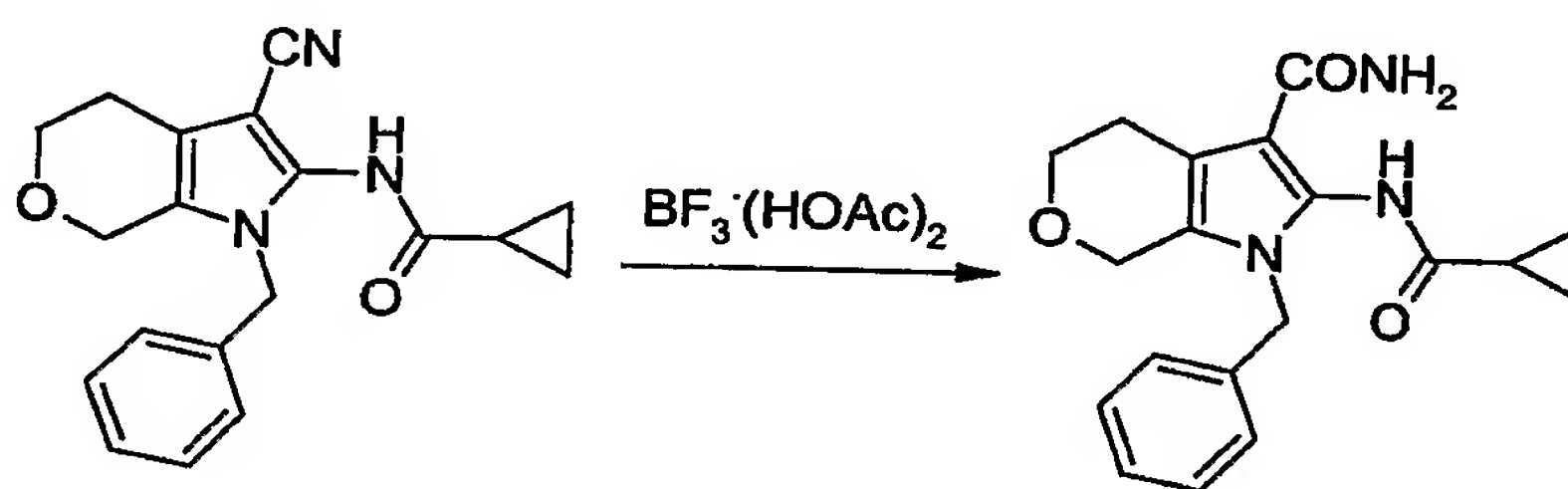
- 1-Benzyl-2-Amino-3-Cyano-4,7-dihydro-5H-pyrrolo[2,3-c]pyrane (4.1 mmol) and cyclopropylcarbonyl chloride (4.9 mmol) were dissolved in 10 mL of abs. THF. 1.5 mL of diisopropylethylamine were added via syringe, and the mixture was stirred overnight at room temperature. After dilution with 20 mL of water, the aqueous phase was extracted four times with ethylacetate, the organic layer washed once with water, 20 dried over sodium sulfate and the solvents evaporated. Recrystallization of the crude material from hot ethanol gave the desired product.

- 25 **Preparation of 2-[(Cyclopropanecarbonyl)amino]-4,7-dihydro-5H-furo[2,3-c]pyrane-3-carboxamide**



2-(Cyclopropanecarbonyl-amino)-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane (4.3 mmol), 1 mL of water, and 7 mL of boron trifluoride-acetic acid complex are heated at 120 °C for 10 minutes. After cooling, the reaction mixture is treated with 50 mL of 6 N sodium hydroxide solution, the aqueous mixture is extracted with ethylacetate, dried over sodium sulfate and the solvents evaporated. The crude material can be recrystallized from hot ethanol.

10 Preparation of **1-Benzyl-2-[(Cyclopropanecarbonyl)amino]-4,7-dihydro-5H-pyrrolo[2,3-c]pyrane-3-carboxamide**



15

1-Benzyl-2-(Cyclopropanecarbonyl-amino)-3-Cyano-4,7-dihydro-5H-pyrrolo[2,3-c]pyrane (4 mmol), 1 mL of water, and 7 mL of boron trifluoride-acetic acid complex are heated at 120 °C for 10 minutes. After cooling, the reaction mixture is treated with 50 mL of 6 N sodium hydroxide solution, the aqueous mixture is extracted with ethylacetate, dried over sodium sulfate and the solvents evaporated. The crude material is recrystallized from hot ethanol.

25 Biochemical methods and experiments

In the following documents, background information is given with regard to the methods, microorganisms and enzymes used according to the present invention: Peirs et al., *A serine/threonine protein kinase from Mycobacterium tuberculosis*, Eur. J. Biochem., Mar 1, 244(2), 604-612 (1997); Arruda et al., *Cloning of an M. tuberculosis DNA fragment associated with entry and survival inside cells*, Science 261, 1454-1457 (1993); Wieles et al., *Increased intracellular survival of Mycobacterium smegmatis containing the Mycobacterium leprae thioredoxin-thioredoxin reductase gene*, Infect Immun. 65(7), 2537-2541 (1997); Zahrt, *Mycobacterium tuberculosis signal transduction system required for persistent infections*, Proc. Natl. Acad. Sci. 98 (22), 12706-12711 (2001); and Mundayoor et al.,

Identification of genes involved in the resistance of mycobacteria to killing by macrophages, Ann. N. Y. Acad. Sci. 730, 26-36 (1994).

Bacterial strains and culture conditions

- 5 *Mycobacterium smegmatis* was grown in Middlebrook 7H9 medium (supplier: Difco), supplemented with 10% ADC (Difco), 0.05% Tween-80 and 0.5% glycerol. *E. coli* was cultivated in LB- or TB-broth without any additional ingredients. Cloning, mutagenesis and expression of PknG and other mycobacterial kinases was done as described by Koul et. al. (Serine/threonine kinases, PknG and PknF of
- 10 *Mycobacterium tuberculosis*: characterisation and localisation. Microbiology, 2001, 147, 2307-23142001).

GST-fusion protein purification

- 15 Purification of GST-fusion proteins was done as described previously by Koul et. al. (Serine/threonine kinases, PknG and PknF of *Mycobacterium tuberculosis*: characterisation. and localisation. Microbiology, 2001,147, 2307-.23142001). *E. coli* BL21 cultures containing the respective plasmids were grown overnight in TB-broth. After IPTG induction, the suspensions were incubated for another 16 hours at room
- 20 temperature. The bacteria were harvested by centrifugation, resuspended in 1x PBS and lysed by sonification. After addition of Triton X-100 (1% final concentration) and subsequent clarifying of the lysates the GST-fusion proteins were purified by addition of GST-sepharose following PBS washes. The proteins were eluted with a buffer containing 50 mM glutathion, 20 mM Tris (pH 8.0), 0.1 M NaCl, 0.1 M Triton X-100
- 25 and 1 mM DTT. Thereafter, the eluates were dialysed in 20 mM HEPES (pH 7.5) and 30 % glycerol.

Determination of protein kinase activity

- 30 The activity of all protein serine threonine kinases from *Mycobacterium tuberculosis* was determined by addition of myelin basic protein as a substrate in an *in vitro* kinase assay. The buffer conditions were as follows: 20 mM HEPES (pH 7.5), 20 mM MgCl₂, and 5 mM MnCl₂, for all kinases except PknI, PknJ, and PknL. These protein kinases required lower salt concentrations, namely 1 mM MgCl₂, and 1 mM MnCl₂.
- 35 The optimum ATP concentration for each kinase was determined by titration of ATP in a range between 0.0033 µM and 100 µM. The inhibitor studies were performed with ATP concentrations similar to the Michaelis constant (K_m) for ATP. Further, the role of PknG in pathogenesis of mycobacteria in cellular infection model was analysed.

Infection of macrophage cells with recombinant *Mycobacterium smegmatis*

- Mycobacterium smegmatis*, electroporated with either vector alone or mycobacterial expression vector containing PknG (wild type) or PknG-K181M (Mutant), was cultured for 2 days in Middlebrook 7H9 medium containing 0.05% Tween-80 and 0.5% glycerol. Bacteria were pelleted at 1500 x g for 3 minutes by centrifugation and resuspended by vigorous agitating (Vortex) in Dulbecco's modified Eagle's medium (DMEM, GIBCO-BRL, Gaithersburg, USA) containing 5 % fetal bovine serum (FBS) for infecting murine macrophage cell line RAW (American Type Culture Collection No. 91B-71). This yielded a bacterial supernatant consisting mostly of single mycobacterial cells as observed by acid fast staining. Under the assumption that an optical density (O.D.) of 0.1 at 650 nm equals to 10^8 CFU/ml (see in this respect Wei et al., "Identification of a *Mycobacterium tuberculosis* Gene that enhances survival of *M. smegmatis* in Macrophages", J. Bacteriol. 182, 377-384 (2000)), the O.D. of *Mycobacterium smegmatis* cell suspension was measured and diluted to 5×10^6 CFU/ml in DMEM containing 5 % FBS. Viable counts were performed on Middlebrook 7H10 medium.
- The RAW cell line was maintained in DMEM medium supplemented with 10 % FBS. The survival assay for recombinant *Mycobacterium smegmatis* was performed as described by Wei et al., cited above. RAW cells were plated in a 24 well tissue culture plate (4×10^5 cells/well) and incubated overnight in 5 % CO₂ at 37°C. The inoculum (1 ml) containing 5×10^6 recombinant *Mycobacterium smegmatis* was added to achieve multiplicities of infection (moi) of 10. The plate was incubated for 2 hours at 37°C in 5 % CO₂. The infected monolayers were washed twice with warm DMEM and treated with 2 % FCS containing 200 µg of amikacin/ml for 1 hour at 37°C to kill extracellular *M. smegmatis*. The cells were again washed twice with warm DMEM and further incubated in DMEM containing 20 µg of amikacin. This time point was considered 0 hours of infection. The 24 hours infected monolayer was incubated with 20 µg of amikacin/ml to prevent extracellular growth of any bacteria released by premature lysis of infected RAW cells. Cells were washed twice with warm DMEM before lysis was effected by addition of a 0.1 % SDS solution and vigorously pipeting several times to ensure lysis of cells and release of surviving bacteria. The lysates were diluted in 7H9 broth and plated onto 7H10 agar plates and CFU were counted after incubation at 37°C for 4 to 5 days.

Validation of mycobacterial kinase as a mycobacterial virulence gene

5 *Mycobacterium smegmatis* was electroporated either with wildtype or mutant kinase (which exerts no kinase activity) or vector control. Mouse macrophage cell line (RAW) was infected with the various recombinant *M. smegmatis* expressing either

10 pknG wild type or PknG K/M mutant or vector alone. After infection, the cells were lysed at different time points and the amount of intracellular bacteria was analysed. As can be seen from Fig. 1, after one hour postinfection the amount of bacteria recovered from macrophages infected with *M. smegmatis* expressing PknG wild type or K/M mutant or vector alone was the same. This shows that the recombinant *M. smegmatis* strains were internalised with equal efficiency. However, after 24 hour postinfection the amount of *M. smegmatis* transformed with the vector control or the

15 mutant kinase was substantially decreased within macrophages. This shows an efficient clearance or degradation of the the *M. smegmatis* expressing vector alone or PknG K/M mutant by the lysosomal degradation pathway with in the macrophages. But in contrast, after 24 hrs an approximately tenfold increased amount of *M. smegmatis* survived within the cells expressing wildtype PknG compared to the mutant. This clearly demonstrates that the kinase activity of PknG increases the

20 intracellular survival of *M. smegmatis* within macrophages and as such makes PknG an important virulence factor of mycobacteria. Consequently, the kinase is a promising target for recognising, monitoring, and controlling therapy of various diseases.

25

Screening for inhibitors of PknG

A search was conducted for specific molecules inhibiting the target kinase (PknG) of *Mycobacterium tuberculosis*. In a kinase platform a suitable substrate was identified

30 and an *in vitro* assay was adapted to high throughput screening. The PknG kinase inhibitors were routinely tested under optimised assay conditions: 20 mM Hepes (pH 7.5), 1.8 μ M ATP, 1 mM DTT, 10 mM MnCl_2 . Subsequently, a library comprising 55.000 compounds using the established *in vitro* kinase assay was screened. Table 1 shows the half-maximal inhibition constant (IC_{50}) values of the compounds 2-

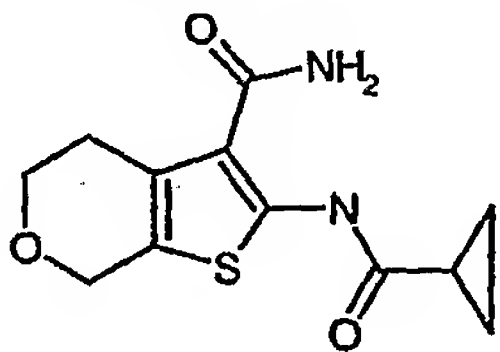
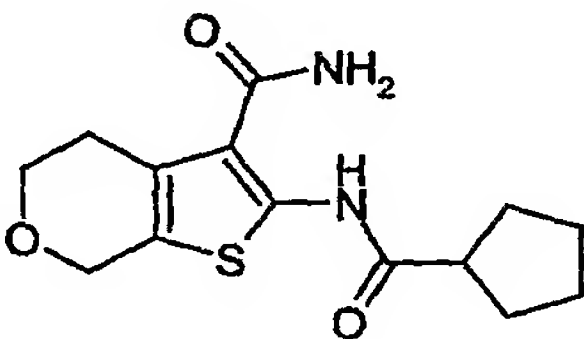
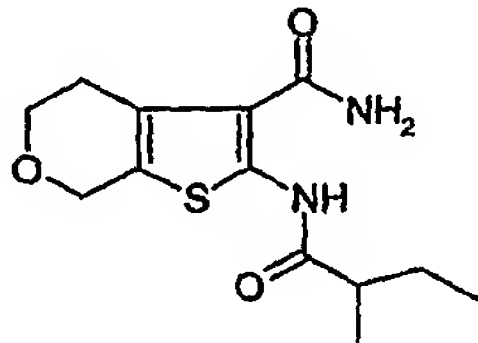
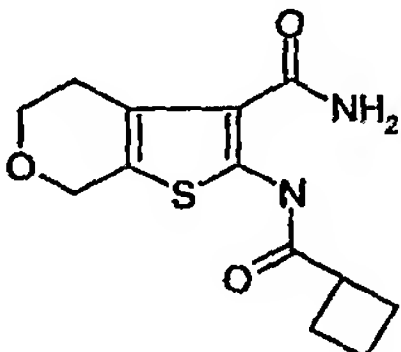
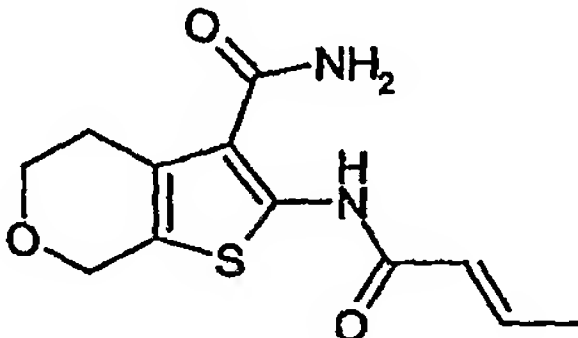
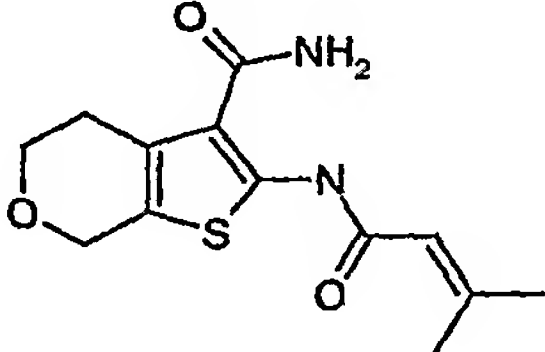
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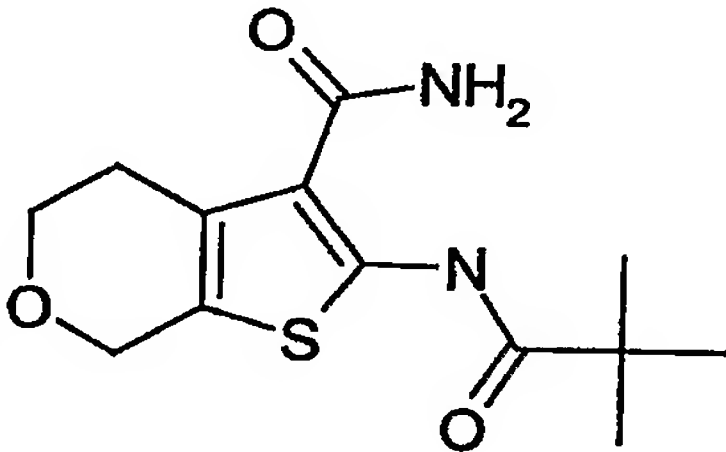
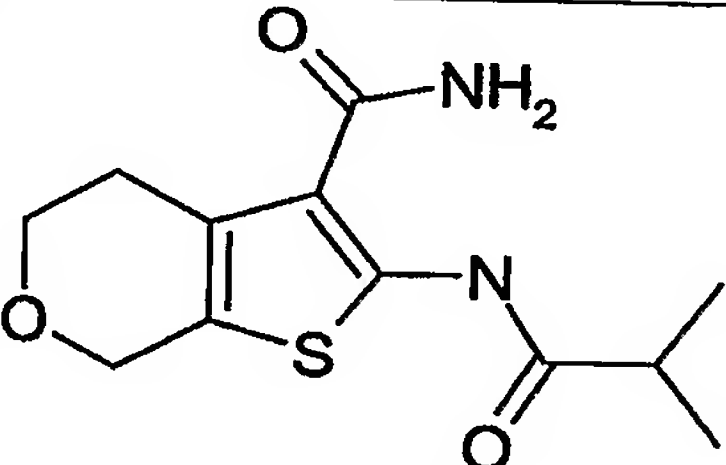
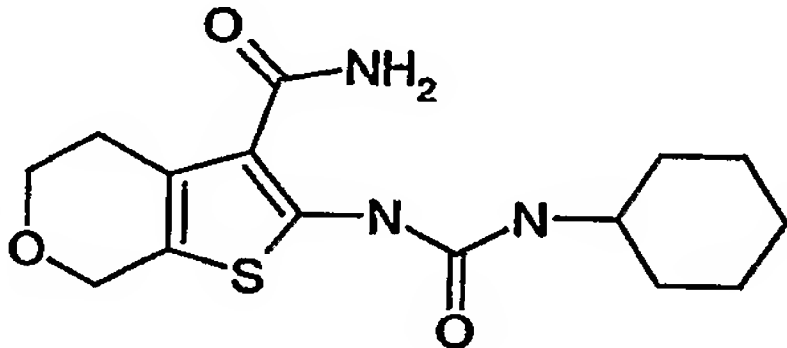
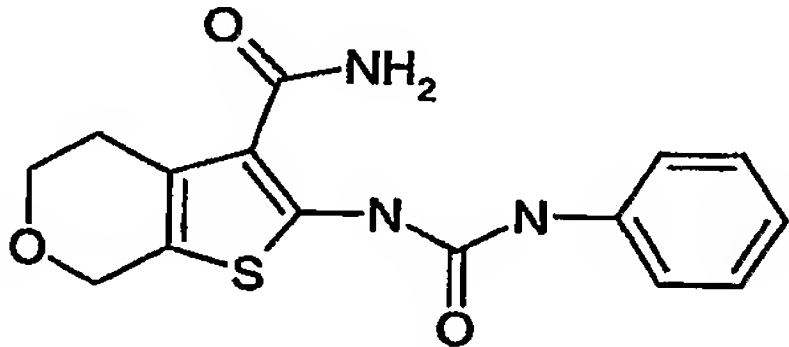
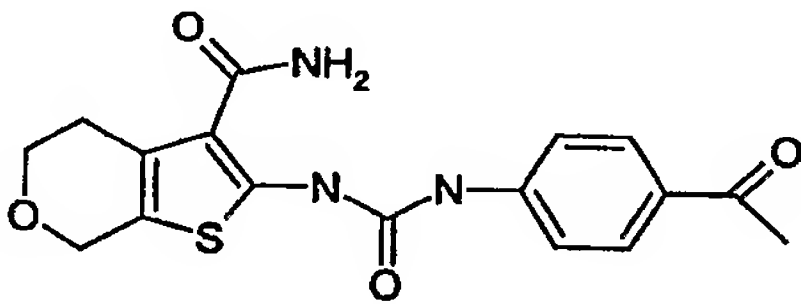
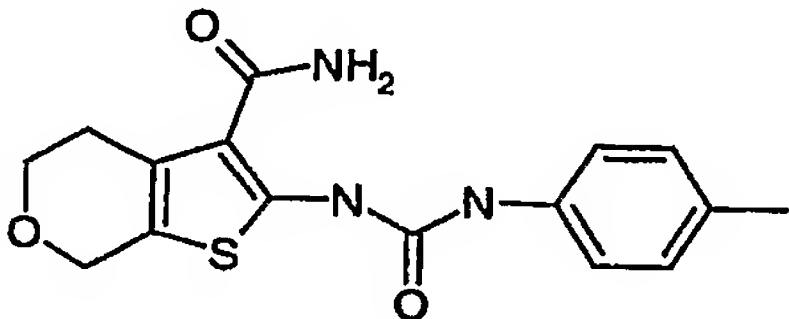
- amide (Compound 4), 2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 6), 2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 7), 2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 8), 2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 10), 2-(3-Cyclohexyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 18), 2-(3-Phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 19), 2-[3-(4-Acetyl-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 20), 2-(3-p-Tolyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 21) and 2-[3-(4-Fluoro-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 22) for inhibiting mycobacterial PknG .
- 15 As is evident from Table I, compound 1, compound 18, compound 19, compound 20, compound 21 and compound 22 are the most effective compounds of those tested in inhibiting the activity of protein serine/threonine kinase G of *M. tuberculosis*, compound 1, compound 18, compound 19, compound 20, compound 21 and compound 22 having IC_{50} values between 0,19 μ M and 0,71 μ M. With compounds 2,
- 20 3, 4, 6, 7, 8, and 10 satisfactory results were also obtained, the compounds having IC_{50} -values, between about 2 μ m and up to about 70 μ m.

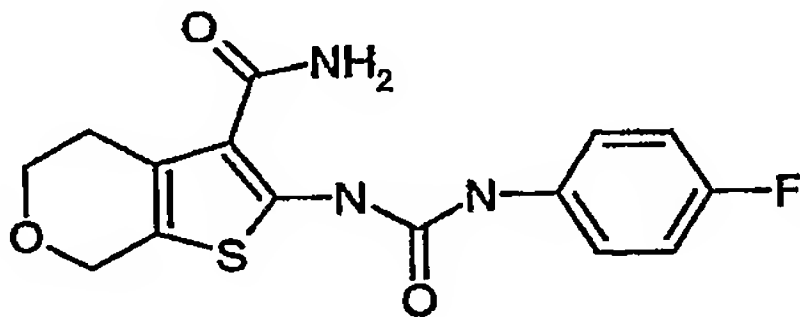
Table I

Inhibitory effect on mycobacterial protein kinase G (PknG) of selected compounds according the present invention

5

Compound No.	Structure	Inhibition of PknG (IC ₅₀ , [μM])
Compound 1: 2-(Cyclopropanecarbonyl- amino)-4,7-dihydro-5H- thieno[2,3-c]pyran-3- carboxylic acid amide		0.49
Compound 2: 2-(Cyclopentanecarbonyl- amino)-4,7-dihydro-5H- thieno[2,3-c]pyran-3- carboxylic acid amide		3,42
Compound 3: 2-(2-Methyl-butrylamino)- 4,7-dihydro-5H-thieno[2,3- c]pyran-3-carboxylic acid amide		69,2
Compound 4: 2-(Cyclobutanecarbonyl- amino)-4,7-dihydro-5H- thieno[2,3-c]pyran-3- carboxylic acid amide		2.17
Compound 6: 2-But-2-enoylamino-4,7- dihydro-5H-thieno[2,3-c] pyran-3-carboxylic acid amide		2,26
Compound 7: 2-(3-Methyl-but-2- enoylamino)-4,7-dihydro- 5H-thieno[2,3-c]pyran-3- carboxylic acid amide		2,64

Compound No.	Structure	Inhibition of PknG (IC ₅₀ , [μM])
Compound 8: 2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		57,3
Compound 10: 2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		20,43
Compound 18: 2-(3-Cyclohexyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		0,71
Compound 19: 2-(3-Phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		0,21
Compound 20: 2-[3-(4-Acetyl-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		0,35
Compound 21: 2-(3-p-Tolyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		0,21

Compound No.	Structure	Inhibition of PknG (IC ₅₀ , [μM])
Compound 22: 2-[3-(4-Fluoro-phenyl)- ureido]-4,7-dihydro-5H- thieno[2,3-c]pyran-3- carboxylic acid amide		0,19

Secretion of PknG outside the bacterial cell

- 5 In the following it is demonstrated that PknG is secreted outside the cell into the culture supernatant by mycobacterial cells.
- 10 1) PknG and ESAT (a secretory protein that acts as a positive control) are cloned in BamH1 site of pYUB 2401. This vector contains the promoter for hsp60. A in-frame fusion with the start of hsp60 and phoA at the C-terminus by cloning into the BamH1 site. The vector is kanamycin resistant. After cloning PknG and ESAT in pYUB2401 they were electroporated in *M. smegmatis* and the colonies were grown on LB plates with 40μg of 5-bromo-4-chloro-3-indoylphosphate (BCIP) and with 20 μg of kanamycin used for screening.
- 15 PhoA fusion proteins that are exported beyond cytoplasm are enzymatically active and capable of hydrolysing the BCIP, the chromogenic substrate of PhoA to produce the blue colonies.
- 20 2) *M. smegmatis* strains containing either
- 1) ESAT-PhoA
 - 2) PknG-PhoA or
 - 3) PhoA alone
- were grown in 7H9 medium with kanamycin to saturation for 5-6 days and then
- 25 diluted to the final optical density (O.D.) of 0.005 at 600 nm.
- 3) These cultures were then grown for 40 hours at 37 °C. The OD₆₀₀ of each strain was measured at the start of the experiment.
- 30 4) A 0.5 ml portion of the cell culture was pelleted and resuspended in equal volume 1 M Tris (pH. 8.0).

- 5) Then 0.1 ml of cells was added to 1.0 ml of 2 mM p-nitrophenyl phosphate plus sodium salt in 1 M Tris (pH 8.0).
- 6) The reaction was incubated in dark at 37 °C until a yellow reaction product was formed.
- 7) Next , 0.1 ml of 1 M K₂HP0₄ was added to terminate the reaction.
- 8) The bacteria were pelleted and the OD₄₂₀ of 1.0 ml of the supernatant was measured.
- 9) Alkaline phosphatase activity units were determined by the following formula:

$$\frac{1000 \times \text{OD}_{420}}{\text{OD}_{600} \times 0.1 \text{ ml volume of cells}}$$

The negative control will be the *M. smegmatis* cells alone and PhoA transfected *M. smegmatis*.

The above method is described in Braunstein M, Griffin TJ IV, Kriakov JI, Friedman ST, Grindley ND, Jacobs WR Jr., „*Mycobacterium tuberculosis* proteins using a *Tn552'phoA in vitro transposition system*”, J Bacteriol. 2000 May;182(10):2732-40.

The result of the above-mentioned experiment shows that PknG is a secretory protein that is secreted outside the mycobacterial cells. Fig. 3 shows the alkaline phosphatase secretions assay for PknG for different PhoA fusion constructs. The secreted PknG can phosphorylate host cell proteins that might be critical in survival of mycobacterium in host cells.

Selectivity panel data:

Table II shows the inhibitory effect of selected compounds according to the present invention on the activity of certain protein kinases. The activity of these protein kinases is depicted as % inhibition in the presence of 10 μ M of compound in comparison to DMSO (0% inhibition).

Table II:

Selectivity panel data (% inhibition) of selected compounds according to the present invention

	PDGF-R	c-kit	GSK-3β	CDK 1	SRPK 1
Compound 1	14	55	38	4	n.a.
Compound 18	20	54	27	50	6
Compound 19	45	73	23	58	68
Compound 20	38	70	14	40	69
Compound 21	25	62	11	29	55
Compound 22	54	64	22	64	56

n.a.: not available

These data show, that compounds according to the present invention, do have a good inhibitory effect on the protein kinase activity of various protein kinases, such as PDGF-R, c-kit, GSK-3 β , CDK 1 and SRPK 1.

The % inhibition values for certain protein kinases shown above were measured according to one of the protocols which are described below. The IC₅₀-value of compounds according to the present invention for inhibiting kinases like for example PDGF-R, c-kit or GSK3- β were also measured according to the protocols described in the following:

c-Kit-Assay

- Reaction Volume: 40 μ l
 Reaction Time: 60 min
 5 Reaction Temperature: room temperature
 Assay Plate: 96 well U bottom plate (Greiner, 650161)
 MultiScreen-PH Plate: 96 well MAPH Filter Plates (Millipore, MAPHNOB50)
 Filter Washing Solution: 0.75% H_3PO_4
 Szintillation Liquid: Supermix Liquid Szintillator (PerkinElmer, 1200-439)

10

Controls:

- Negative Control (C-): 100 mM EDTA, no Inhibitor
 15 Positive Control (C+): no Inhibitor

Reaction Buffer:

- 20 mM Hepes, pH 7.5
 20 10 mM MgCl_2
 1 mM DTT
 0.01% Tween20

25 Final Assay Concentrations:

- Kinase: Use kinase conc. yielding 10% ATP turn over.
 ATP: 16.9 μ M
 Adenosine 5'-[γ - ^{33}P]triphosphate: 12.5 μ Ci/ml (Amersham Biosciences, BF1000)
 30 Substrate: Myelin Basic Protein, 30 μ M (Invitrogen, 13228-010)

Pipetting Sequence:

- 35 1) Add 10 μ l 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate

- 2) Add 10 μ l 4 fold concentrated inhibitor in 4% DMSO in H₂O to each well except to C- and C+ wells
- 3) Add 10 μ l 4% DMSO in H₂O to C- and C+ wells
- 4) Add 10 μ l 500 mM EDTA in H₂O to C- wells
- 5) Add 10 μ l 50 μ Ci/ml Adenosine 5'-[γ -³³P]triphosphate in H₂O to each well
- 6) Add 10 μ l 4 fold concentrated kinase in Reaction Buffer to each well
- 7) Incubate 1hr at room temperature
- 8) Add 10 μ l 50 mM EDTA in H₂O to each well except to C- wells
- 9) Prepare MAPH plates by adding 200 μ l 0.75% H₃PO₄ to each well
- 10) Exhaust 0.75% H₃PO₄ using Millipore vacuum station
- 11) Add 60 μ l 0.75% H₃PO₄ to each well of MAPH Filter Plate
- 12) Transfer 30 μ l sample per well from Assay Plate to corresponding well of MAPH Filter Plate
- 13) Incubate 30 min at room temperature
- 14) Wash each well of MAPH Filter Plates 3x with 200 μ l 0.75% H₃PO₄ using Millipore vacuum station
- 15) Add 20 μ l Szintillation Liquid to each well of MAPH Filter Plate
- 16) Seal MAPH Filter Plate
- 17) Store MAPH Filter Plate 30 min in darkness
- 18) Quantify radioactivity

PDGF-R assay:

- | | | |
|----|--------------------------|--|
| 25 | Reaction Volume: | 40 μ l |
| | Reaction Time: | 60 min |
| | Reaction Temperature: | room temperature |
| | Assay Plate: | 96 well U bottom plate (Greiner, 650161) |
| | MultiScreen-PH Plate: | 96 well MAPH Filter Plates (Millipore, MAPHNOB50) |
| 30 | Filter Washing Solution: | 0.75% H ₃ PO ₄ |
| | Szintillation Liquid: | Supernix Liquid Szintillator (PerkinElmer, 1200-439) |

Controls:

- | | | |
|----|------------------------|---------------------------|
| 35 | Negative Control (C-): | 100 mM EDTA, no Inhibitor |
| | Positive Control (C+): | no Inhibitor |

Reaction Buffer:

20 mM Tris, pH 7.5
 10 mM MgCl₂
 5 0.4 mM MnCl₂
 1 mM DTT
 0.01% Brij35

Final Assay Concentrations:

10 Kinase: Use kinase conc. yielding 10% ATP turn over.
 ATP: 16.8 μ M
 Adenosine 5'-[γ -³³P]triphosphate: 12.5 μ Ci/ml (Amersham Biosciences, BF1000)
 15 Substrate: Myelin Basic Protein, 20 μ M (Invitrogen, 13228-010)

Pipetting Sequence:

- 20 1) Add 10 μ l 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate
- 2) Add 10 μ l 4 fold concentrated inhibitor in 4% DMSO in H₂O to each well except to C- and C+ wells
- 3) Add 10 μ l 4% DMSO in H₂O to C- and C+ wells
- 4) Add 10 μ l 500 mM EDTA in H₂O to C- wells
- 25 5) Add 10 μ l 50 μ Ci/ml Adenosine 5'-[γ -³³P]triphosphate in H₂O to each well
- 6) Add 10 μ l 4 fold concentrated kinase in Reaction Buffer to each well
- 7) Incubate 1hr at room temperature
- 8) Add 10 μ l 50 mM EDTA in H₂O to each well except to C- wells
- 9) Prepare MAPH plates by adding 200 μ l 0.75% H₃PO₄ to each well
- 30 10) Exhaust 0.75% H₃PO₄ using Millipore vacuum station
- 11) Add 60 μ l 0.75% H₃PO₄ to each well of MAPH Filter Plate
- 12) Transfer 30 μ l sample per well from Assay Plate to corresponding well of MAPH Filter Plate
- 13) Incubate 30 min at room temperature

- 14) Wash each well of MAPH Filter Plates 3x with 200 μ l 0.75% H_3PO_4 using Millipore vacuum station
- 15) Add 20 μ l Szintilation Liquid to each well of MAPH Filter Plate
- 16) Seal MAPH Filter Plate
- 5 17) Store MAPH Filter Plate 30 min in darkness
- 18) Quantify radioactivity

GSK-3 β assay

- | | | |
|----|--------------------------|--|
| 10 | Reaction Volume: | 40 μ l |
| | Reaction Time: | 60 min |
| | Reaction Temperature: | room temperature |
| | Assay Plate: | 96 well U bottom plate (Greiner, 650161) |
| | MultiScreen-PH Plate: | 96 well MAPH Filter Plates (Millipore, MAPHNOB50) |
| 15 | Filter Washing Solution: | 0.75% H_3PO_4 |
| | Szintilation Liquid: | Supremix Liquid Szintillator (PerkinElmer, 1200-439) |

Controls:

- | | | |
|----|------------------------|---------------------------|
| 20 | Negative Control (C-): | 100 mM EDTA, no Inhibitor |
| | Positive Control (C+): | no Inhibitor |

Reaction Buffer:

- | | |
|----|----------------------|
| 25 | 20 mM Mops, pH 7.0 |
| | 2 mM MgCl_2 |
| | 1 mM DTT |
| | 0.01% Tween20 |

30 Final Assay Concentrations:

- | | | |
|----|--|--|
| | Kinase: | Use kinase conc. yield. 10% ATP turn over. |
| | ATP: | 7.76 μ M |
| | Adenosine 5'-[γ - ^{33}P]triphosphate: | 12.5 μ Ci/ml (Amersham Biosciences, BF1000) |
| 35 | Substrate: | Phospho-Glycogen Synthase Pept.2, 10 μ M (upstate, 12-241) |

Pipetting Sequence:

- | | |
|----|---|
| 5 | 1) Add 10 µl 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate |
| | 2) Add 10 µl 4 fold concentrated inhibitor in 4% DMSO in H ₂ O to each well except to C- and C+ wells |
| | 3) Add 10 µl 4% DMSO in H ₂ O to C- and C+ wells |
| 10 | 4) Add 10 µl 500 mM EDTA in H ₂ O to C- wells |
| | 5) Add 10 µl 50 µCi/ml Adenosine 5'-[γ- ³³ P]triphosphate in H ₂ O to each well |
| | 6) Add 10 µl 4 fold concentrated kinase in Reaction Buffer to each well |
| | 7) Incubate 1hr at room temperature |
| | 8) Add 10 µl 50 mM EDTA in H ₂ O to each well except to C- wells |
| 15 | 9) Prepare MAPH plates by adding 200 µl 0.75% H ₃ PO ₄ to each well |
| | 10) Exhaust 0.75% H ₃ PO ₄ using Millipore vacuum station |
| | 11) Add 60 µl 0.75% H ₃ PO ₄ to each well of MAPH Filter Plate |
| | 12) Transfer 30 µl sample per well from Assay Plate to corresponding well of MAPH Filter Plate |
| 20 | 13) Incubate 30 min at room temperature |
| | 14) Wash each well of MAPH Filter Plates 3x with 200 µl 0.75% H ₃ PO ₄ using Millipore vacuum station |
| | 15) Add 20 µl Szintilation Liquid to each well of MAPH Filter Plate |
| | 16) Seal MAPH Filter Plate |
| 25 | 17) Store MAPH Filter Plate 30 min in darkness |
| | 18) Quantify radioactivity |

CDK 1 assay

- | | | |
|----|--------------------------|---|
| 30 | Reaction Volume: | 40 µl |
| | Reaction Time: | 60 min |
| | Reaction Temperature: | room temperature |
| | Assay Plate: | 96 well U bottom plate (Greiner, 650161) |
| | MultiScreen-PH Plate: | 96 well MAPH Filter Plates (Millipore, MAPHNOB50) |
| 35 | Filter Washing Solution: | 0.75% H ₃ PO ₄ |

Szintillation Liquid: Supermix Liquid Szintillator (PerkinElmer, 1200-439)

Controls:

5
Negative Control (C-): 100 mM EDTA, no Inhibitor
Positive Control (C+): no Inhibitor

10 Reaction Buffer:

20 mM Mops, pH 7.0

10 mM MgCl₂

1 mM DTT

15 0.01% Brij35

Final Assay Concentrations:

20 Kinase: Use kinase conc. yielding 10% ATP turn over.
ATP: 27 μ M
Adenosine 5'-[γ -³³P]triphosphate: 12.5 μ Ci/ml (Amersham Biosciences, BF1000)
Substrate: PKTPKKAKKL-NH232 μ M (Jerini)

25

Pipetting Sequence:

- 1) Add 10 μ l 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate
- 30 2) Add 10 μ l 4 fold concentrated inhibitor in 4% DMSO in H₂O to each well except to C- and C+ wells
- 3) Add 10 μ l 4% DMSO in H₂O to C- and C+ wells
- 4) Add 10 μ l 500 mM EDTA in H₂O to C- wells
- 5) Add 10 μ l 50 μ Ci/ml Adenosine 5'-[γ -³³P]triphosphate in H₂O to each well
- 35 6) Add 10 μ l 4 fold concentrated kinase in Reaction Buffer to each well
- 7) Incubate 1hr at room temperature
- 8) Add 10 μ l 50 mM EDTA in H₂O to each well except to C- wells
- 9) Prepare MAPH plates by adding 200 μ l 0.75% H₃PO₄ to each well

- 10) Exhaust 0.75% H_3PO_4 using Millipore vacuum station
- 11) Add 60 μl 0.75% H_3PO_4 to each well of MAPH Filter Plate
- 12) Transfer 30 μl sample per well from Assay Plate to corresponding well of MAPH Filter Plate
- 5 13) Incubate 30 min at room temperature
- 14) Wash each well of MAPH Filter Plates 3x with 200 μl 0.75% H_3PO_4 using Millipore vacuum station
- 15) Add 20 μl Szintilation Liquid to each well of MAPH Filter Plate
- 16) Seal MAPH Filter Plate
- 10 17) Store MAPH Filter Plate 30 min in darkness
- 18) Quantify radioactivity

SRPK 1 assay

- | | | |
|----|--------------------------|--|
| 15 | Reaction Volume: | 40 μl |
| | Reaction Time: | 60 min |
| | Reaction Temperature: | room temperature |
| | Assay Plate: | 96 well U bottom plate (Greiner, 650161) |
| | MultiScreen-PH Plate: | 96 well MAPH Filter Plates (Millipore, MAPHNOB50) |
| 20 | Filter Washing Solution: | 0.75% H_3PO_4 |
| | Szintilation Liquid: | Supremix Liquid Szintillator (PerkinElmer, 1200-439) |

Controls:

- | | | |
|----|------------------------|---------------------------|
| 25 | Negative Control (C-): | 100 mM EDTA, no Inhibitor |
| | Positive Control (C+): | no Inhibitor |

Reaction Buffer:

- | | |
|----|------------------------|
| 30 | 20 mM Hepes, pH 7.5 |
| | 0.4 mM MgCl_2 |
| | 0.4 mM MnCl_2 |
| | 1 mM DTT |
| | 0.01% Tween20 |

Final Assay Concentrations:

	Kinase:	Use kinase conc. yielding 10% ATP turn over.
5	ATP:	0.56 μ M
	Adenosine 5'-[γ - 33 P]triphosphate:	12.5 μ Ci/ml (Amersham Biosciences, BF1000)
	Substrate:	PKC epsilon peptide, 3.1 μ M (Biomol, P-155)

10 Pipetting Sequence:

- 1) Add 10 μ l 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate
- 2) Add 10 μ l 4 fold concentrated inhibitor in 4% DMSO in H₂O to each well except to C- and C+ wells
- 3) Add 10 μ l 4% DMSO in H₂O to C- and C+ wells
- 4) Add 10 μ l 500 mM EDTA in H₂O to C- wells
- 5) Add 10 μ l 50 μ Ci/ml Adenosine 5'-[γ - 33 P]triphosphate in H₂O to each well
- 6) Add 10 μ l 4 fold concentrated kinase in Reaction Buffer to each well
- 7) Incubate 1hr at room temperature
- 8) Add 10 μ l 50 mM EDTA in H₂O to each well except to C- wells
- 9) Prepare MAPH plates by adding 200 μ l 0.75% H₃PO₄ to each well
- 10) Exhaust 0.75% H₃PO₄ using Millipore vacuum station
- 11) Add 60 μ l 0.75% H₃PO₄ to each well of MAPH Filter Plate
- 12) Transfer 30 μ l sample per well from Assay Plate to corresponding well of MAPH Filter Plate
- 13) Incubate 30 min at room temperature
- 14) Wash each well of MAPH Filter Plates 3x with 200 μ l 0.75% H₃PO₄ using Millipore vacuum station
- 15) Add 20 μ l Szintillation Liquid to each well of MAPH Filter Plate
- 16) Seal MAPH Filter Plate
- 17) Store MAPH Filter Plate 30 min in darkness
- 18) Quantify radioactivity

According to one of the protocols described above, for 2-(Cyclopropanecarbonylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (compound 1) the following IC₅₀-values for inhibiting the protein kinases c-kit, GSK-3 β And PDGF-R were obtained:

5

Inhibition of c-kit : 5.4 μ M (IC₅₀),
 Inhibition of GSK-3 β : 27 μ M (IC₅₀),
 Inhibition of PDGF-R: 90 μ M (IC₅₀).

10

General kinase assay:

Table III shows all currently known protein kinases. The inhibitory effect of compounds according to the present invention on the activity of these protein kinases may be measured according to the following protocol:

15

Reaction Volume: 40 μ l
 Reaction Time: 60 min
 Reaction Temperature: room temperature
 20 Assay Plate: 96 well U bottom plate (Greiner, 650161)
 MultiScreen-PH Plate: 96 well MAPH Filter Plates (Millipore, MAPHNOB50)
 Filter Washing Solution: 0.75% H₃PO₄
 Szintillation Liquid: Supermix Liquid Szintillator (PerkinElmer, 1200-439)

25 Controls:

Negative Control (C-): 100 mM EDTA, no Inhibitor
 Positive Control (C+): no Inhibitor

30 Reaction Buffer:

20 mM Tris, pH 7.5
 10 mM MgCl₂
 1 mM DTT

35

Final Assay Concentrations:

Kinase: Use kinase conc. yielding 10% ATP turn over.
 ATP: 1 μ M
 5 Adenosine 5'-[γ - 33 P]triphosphate: 12.5 μ Ci/ml (Amersham Biosciences, BF1000)
 Substrate: Myelin Basic Protein, 10 μ M (Invitrogen, 13228-010)

Pipetting Sequence:

10

- 1) Add 10 μ l 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate
- 2) Add 10 μ l 4 fold concentrated inhibitor in 4% DMSO in H₂O to each well except to C- and C+ wells

15

- 3) Add 10 μ l 4% DMSO in H₂O to C- and C+ wells
- 4) Add 10 μ l 500 mM EDTA in H₂O to C- wells
- 5) Add 10 μ l 50 μ Ci/ml Adenosine 5'-[γ - 33 P]triphosphate in H₂O to each well
- 6) Add 10 μ l 4 fold concentrated kinase in Reaction Buffer to each well
- 7) Incubate 1hr at room temperature

20

- 8) Add 10 μ l 50 mM EDTA in H₂O to each well except to C- wells
- 9) Prepare MAPH plates by adding 200 μ l 0.75% H₃PO₄ to each well
- 10) Exhaust 0.75% H₃PO₄ using Millipore vacuum station
- 11) Add 60 μ l 0.75% H₃PO₄ to each well of MAPH Filter Plate
- 12) Transfer 30 μ l sample per well from Assay Plate to corresponding well of MAPH Filter Plate

25

- 13) Incubate 30 min at room temperature
- 14) Wash each well of MAPH Filter Plates 3x with 200 μ l 0.75% H₃PO₄ using Millipore vacuum station

- 15) Add 20 μ l Szintillation Liquid to each well of MAPH Filter Plate

30

- 16) Seal MAPH Filter Plate
- 17) Store MAPH Filter Plate 30 min in darkness
- 18) Quantify radioactivity

Table III: List of protein kinases

No.	Accession Number	Gene
1	NM_001105	ACVR1 (activin A receptor, type I)
2	NM_004302	ACVR1B (activin A receptor, type IB)
3	NM_145259	ACVR1C, ALK7
4	NM_001616	ACVR2, activin A receptor, type II
5	NM_001106	ACVR2B, activin A receptor, type IIB
6	NM_000020	ACVRL1 (activin A receptor type II-like 1)
7	NM_004612	TGFBF1 (transforming growth factor, beta receptor I (activin A receptor type II-like kinase, 53kD))
8	NM_003242	TGFBF2 (transforming growth factor, beta receptor II)
9	NM_004329	BMPR1A (bone morphogenetic protein receptor, type IA)
10	NM_001203	BMPR1B (bone morphogenetic protein receptor, type IB)
11	NM_001204	BMPR2 (bone morphogenetic protein receptor, type II (serine/threonine kinase))
12	NM_006251	PRKAA1 (protein kinase, AMP-activated, alpha 1 catalytic subunit)
13	NM_006252	PRKAA2 (protein kinase, AMP-activated, alpha 2 catalytic subunit)
14	NM_002929	GRK1; rhodopsin kinase
15	NM_001619	GRK2

No.	Accession Number	Gene
16	NM_005160	GRK3
17	NM_005307 NM_182982	GRK4
18	NM_005308	GRK5
19	NM_002082	GRK6
20	NM_139209	GRK7 (G protein-coupled receptor kinase 7)
21	NM_017572	MKNK2, GPRK7
22	NM_001654	ARAF1 (v-raf murine sarcoma 3611 viral oncogene homolog 1)
23	NM_004333	BRAF (v-raf murine sarcoma viral oncogene homolog B1)
24	NM_002880	RAF1 (v-raf-1 murine leukemia viral oncogene homolog 1)
25	NM_021574 NM_004327	BCR1
26	NM_003656	CAMK1 (calcium/calmodulin-dependent protein kinase I)
27	NM_015981 NM_171825	CAMK2A (calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha)
28	NM_001220	CAMK2B (calcium/calmodulin-dependent protein kinase (CaM kinase) II beta)
29	NM_001221	CAMK2D (calcium/calmodulin-dependent protein kinase (CaM kinase) II delta)
30	NM_020439	CAMK1G (calcium/calmodulin-dependent protein kinase IG)

has STK activity

no kinase domain

No.	Accession Number	Gene
31	NM_001222 NM_172169 NM_172170 NM_172171 NM_172172 NM_172173	CAMK2G (calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma)
32	NM_001744	CAMK4 (calcium/calmodulin-dependent protein kinase IV)
33	NM_001786 NM_033379	CDC2 (cell division cycle 2)
34	NM_001798 NM_052827	CDK2 (cyclin-dependent kinase 2)
35	NM_001258	CDK3 (cyclin-dependent kinase 3)
36	NM_000075 NM_032913	CDK4 (cyclin-dependent kinase 4)
37	NM_004935	CDK5 (cyclin-dependent kinase 5)
38	NM_001259	CDK6 (cyclin-dependent kinase 6)
39	NM_001799	CDK7 (cyclin-dependent kinase 7)
40	NM_001260	CDK8 (cyclin-dependent kinase 8)
41	NM_001261	CDK9 (cyclin-dependent kinase 9 (CDC2-related kinase))
42	NM_003674	CDK10 (cyclin-dependent kinase (CDC2-like) 10)
43	NM_015076	CDK11, DPK
44	NM_004196	CDKL1 (cyclin-dependent kinase-like 1); KKIALRE
45	NM_003948	CDKL2 (cyclin-dependent kinase-like 2); KKIAMRE
46	NM_016508	CDKL3 (cyclin-dependent kinase-like 3); NKIAMRE
47	XM_293029 AX166534 cds: 1-1083	CDKL4, similar to cyclin-dependent kinase-like 1
48	NM_033489 8 transcripts	CDC2L1 (cell division cycle 2-like 1); PITSLRE B
49	NM_024011 NM_033536 9 transcripts	CDC2L1 (cell division cycle 2-like 1); PITSLRE A
50	NM_003718 NM_031267	CDC2L5 (cell division cycle 2-like 5)

No.	Accession Number	Gene
51	NM_006201 NM_033018 NM_033019	PCTK1 (PCTAIRE protein kinase 1)
52	NM_002595	PCTK2 (PCTAIRE protein kinase 2)
53	NM_002596	PCTK3 (PCTAIRE protein kinase 3)
54	NM_012395	PFTK1 (PFTAIRE protein kinase 1)
55	NM_001278	IKK-alpha; CHUK
56	NM_001556 XM_032491	IKK-beta; IKK2
57	NM_001892	CSNK1A1 (casein kinase 1, alpha 1)
58	NM_001893	CSNK1D (casein kinase 1, delta)
59	NM_001894	CSNK1E (casein kinase 1, epsilon)
60	NM_004384	CSNK1G3 (casein kinase 1, gamma 3)
61	NM_001319	CSNK1G2 (casein kinase 1, gamma 2)
62	NM_001895	CSNK2A1 (casein kinase 2, alpha 1)
63	NM_001896	CSNK2A2 (casein kinase 2, alpha prime)
64	NM_022048	CSNK1G1 (casein kinase 1, gamma 1)
65	NM_004071	CLK1 (CDC-like kinase 1)
66	NM_003993 NM_001291	CLK2 (CDC-like kinase 2)
67	NM_003992 NM_001292	CLK3 (CDC-like kinase 3)
68	NM_020666	CLK4 (CDC-like kinase 4)
69	NM_004938	DAPK1 (death-associated protein kinase 1)
70	NM_014326	DAPK2 (death-associated protein kinase 2)
71	NM_001348	DAPK3 (death-associated protein kinase 3)
72	NM_004954 NM_017490	EMK1 (ELKL motif kinase)
73	NM_002746	MAPK3; ERK1

No.	Accession Number	Gene
74	NM_002745	MAPK1, ERK2
75	NM_002748	MAPK6; ERK3
76	NM_002747	MAPK4; ERK3-related
77	NM_002749	MAPK7; ERK5
78	NM_001315 NM_139012 NM_139013 NM_139014	MAPK14; CSBP1
79	NM_002751 NM_138993	MAPK11; p38beta
80	NM_002969	MAPK12; ERK6, p38g
81	NM_002754	MAPK13; p38delta
82	AY065978 AX056411 cds 30-1664 NM_139021	ERK8
83	NM_002750	MAPK8; JNK1
84	NM_002752	MAPK9; JNK2
85	NM_002753	MAPK10; JNK3
86	NM_006712 NM_033015 no kinase domain	FASTK (Fas-activated protein kinase)
87	NM_004579	MAP4K2; GCK
88	NM_019884	GSK3A (glycogen synthase kinase 3 alpha)
89	NM_002093	GSK3B (glycogen synthase kinase 3 beta)
90	NM_002576	PAK1
91	NM_002577	PAK2
92	NM_002578	PAK3
93	NM_005884	PAK4
94	NM_020341	PAK5 (PAK7)
95	NM_020168	PAK6

No.	Accession Number	Gene
96	NM_007181	MAP4K1; HPK1
97	NM_004517	ILK (integrin-linked kinase)
98	NM_001569	IRAK1 (interleukin-1 receptor-associated kinase 1)
99	NM_001570	IRAK2 (interleukin-1 receptor-associated kinase 2)
100	NM_007199	IRAK-M
101	NM_016123	IRAK4
102	NM_006575	MAP4K5
103	NM_002314 NM_016735	LIMK1 (LIM domain kinase 1)
104	NM_005569 NM_016733	LIMK2 (LIM domain kinase 2)
105	NM_000455	STK11; LKB1
106	NM_005906	MAK (male germ cell-associated kinase)
107	NM_002755	MAP2K1; MEK1
108	NM_030662	MAP2K2; MEK2
109	NM_002756	MAP2K3; MEK3
110	NM_003010	MAP2K4; MEK4
111	NM_002757	MAP2K5; MEK5
112	NM_002758 NM_031988	MAP2K6; MEK6
113	NM_005043	MAP2K7; MKK7
114	XM_042066 AF042838	MAP3K1; MEKK1
115	NM_006609	MAP3K2; MEKK2
116	NM_002401	MAP3K3; MEKK3
117	NM_005922 NM_006724	MAP3K4; MEKK4
118	NM_005923	MAP3K5; ASK1

No.	Accession Number	Gene
119	NM_004672	MAP3K6
120	NM_003188	MAP3K7; TAK1
121	NM_005204	MAP3K8; Tpl-2
122	XM_027237 AF251442	MAP3K9; MLK1
123	NM_002446	MAP3K10; MST; MLK2
124	NM_002419	MAP3K11; MLK3
125	NM_006301	MAP3K12; DLK
126	NM_004721	MAP3K13; LZK
127	NM_003954	MAP3K14; NIK
128	AX282911 cds: 1-4080 XM_372199	MAP3K7, similar to MAP/ERK kinase kinase 5; apoptosis signal regulating kinase
129	AX504239 cds: 1-2208 AK122935	MAP3K8
130	NM_015112	MAST205
131	NM_005965	MYLK (myosin, light polypeptide kinase)
132	NM_033118	MYLK2 (myosin light chain kinase 2)
133	NM_005372	MOS (v-mos Moloney murine sarcoma viral oncogene homolog)
134	NM_006282	STK4; MST1
135	NM_006281	STK3; MST2
136	NM_003576	STK24; MST3
137	NM_012224	NEK1 (NIMA (never in mitosis gene a)-related kinase 1)
138	NM_002497	NEK2 (NIMA (never in mitosis gene a)-related kinase 2)
139	NM_002498 Z29067	NEK3 (NIMA (never in mitosis gene a)-related kinase 3)

No.	Accession Number	Gene
140	AX394707 XM_292160	NEK5
141	NM_014397	NEK6 (NIMA (never in mitosis gene a)-related kinase 6)
142	NM_133494 AR130839 (ext 3 non-coding region)	NEK7
143	NM_178170	NEK8, NEK12A
144	NM_033116 AR100127	NEK9
145	AX250157 cds:1-2561 NM_152534	NEK10
146	NM_024800 NM_145910	NEK11
147	NM_003157	STK2
148	NM_005406	ROCK1 (Rho-associated, coiled-coil containing protein kinase 1); p160ROCK
149	NM_004850	ROCK2 (Rho-associated, coiled-coil containing protein kinase 2)
150	NM_007271	STK38; NDR
151	NM_015000	STK38L, NDR2
152	NM_004409	DMPK1 (dystrophia myotonica-protein kinase)
153	XM_290516	DMPK2, HSMDPKIN
154	NM_003607	MRCKalpha (PK428)
155	NM_007174 AX166510 AB023166 (C-Term. Longer)	Citron
156	NM_002613	PDPK1 (3-phosphoinositide dependent protein kinase-1)
157	NM_006213	PHKG1 (phosphorylase kinase, gamma 1)
158	NM_000294	PHKG2 (phosphorylase kinase, gamma 2)
159	NM_002648	PIM1
160	NM_006875	PIM2

No.	Accession Number	Gene
161	AR208686	PIM3
162	NM_014791	KIAA0175
163	NM_002730	PRKACA (protein kinase, cAMP-dependent, alpha)
164	NM_002731	PRKACB (protein kinase, cAMP-dependent, beta)
165	NM_002732	PRKACG (protein kinase, cAMP-dependent, gamma)
166	NM_002742	PRKCM (protein kinase C, mu)
167	NM_002737	PRKCA (protein kinase C, alpha)
168	NM_002738 X07109	PRKCB1 (protein kinase C, beta 1)
169	NM_006254	PRKCD (protein kinase C, delta)
170	NM_005400	PRKCE (protein kinase C, epsilon)
171	NM_002739	PRKCG (protein kinase C, gamma)
172	NM_006255	PRKCH (protein kinase C, eta)
173	NM_002740	PRKCI (protein kinase C, iota)
174	NM_006257	PRKCQ (protein kinase C, theta)
175	NM_002744	PRKCZ (protein kinase C, zeta)
176	NM_002741	PRKCL1 (protein kinase C-like 1)
177	NM_006256	PRKCL2 (protein kinase C-like 2)
178	NM_006258	PRKG1 (protein kinase, cGMP-dependent, type I)
179	NM_006259	PRKG2 (protein kinase, cGMP-dependent, type II); cGKII
180	NM_002759	PRKR (protein kinase, interferon-inducible double stranded RNA dependent)
181	NM_006852	TLK2 (tousled-like kinase 2)

No.	Accession Number	Gene
182	NM_012290	TLK1 (tousled-like kinase 1)
183	NM_005044	PRKX (protein kinase, X-linked)
184	NM_005030	PLK (polo-like kinase)
185	NM_004073	CNK (cytokine-inducible kinase)
186	NM_003913	PRPF4B
187	NM_006742	PSKH1 (protein serine kinase H1)
188	NM_005163	AKT1 (v-akt murine thymoma viral oncogene homolog 1)
189	NM_001626	AKT2 (v-akt murine thymoma viral oncogene homolog 2)
190	NM_005465	AKT3 (v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma))
191	NM_014264	STK18; Sak
192	NM_005627	SGK (serum/glucocorticoid regulated kinase)
193	NM_002376	MARK3 (MAP/microtubule affinity-regulating kinase 3)
194	NM_006374	STK25; YSK1
195	NM_003137	SRPK1 (SFRS protein kinase 1)
196	NM_182692	SRPK2 (SFRS protein kinase 2)
197	NM_003319	Titin
198	NM_003318	TTK protein kinase
199	NM_003384	VRK1 (vaccinia related kinase 1)
200	NM_006296	VRK2 (vaccinia related kinase 2)
201	NM_003390	WEE1
202	NM_018650	MARK1 (MAP/microtubule affinity-regulating kinase 1)
203	NM_003160	STK13; (aurora/IPL 1-like), AIE2, aurora kinase C

No.	Accession Number	Gene
204	NM_004759	MAPKAPK2
205	NM_004635	MAPKAPK3
206	NM_003668	MAPKAPK5
207	NM_005734	HIPK3 (homeodomain interacting protein kinase 3),
208	NM_003503	DYRK6
209	NM_016231	CDC7L1 (CDC7 cell division cycle 7-like 1)
210	NM_003565	NLK
211	NM_014683	ULK1 (unc-51-like kinase 1)
212	AX056454	ULK2 (unc-51-like kinase 2)
213	NM_017886	DKFZP434C131 protein, ULK3
214	NM_053006	hypothetical protein FLJ20574, ULK4
215	NM_003684	STK22B; TSSK2
216	NM_003804	MKNK1 (MAP kinase-interacting serine/threonine kinase 1); MNK1
217	NM_003821	RIPK1 (receptor (TNFRSF)-interacting serine-threonine kinase 1); RIP
218	NM_006871	RIPK2 (receptor-interacting serine-threonine kinase 2); RICK
219	NM_003600	RIPK3 (receptor-interacting serine-threonine kinase 3); RIP3
220	NM_004217	STK6; BTAK, AIK
		STK12; IPL1, aurora kinase B

No.	Accession Number	Gene
221	NM_006549	CAMKK2 (calcium/calmodulin-dependent protein kinase kinase 2, beta)
222	NM_017719	SNRK (SNF-1 related kinase)
223	NM_001433 AF059198	ERN1 (ER to nucleus signalling 1)
224	NM_004336	BUB1 (BUB1 budding uninhibited by benzimidazoles 1 homolog)
225	NM_001211	BUB1B (BUB1 budding uninhibited by benzimidazoles 1 homolog beta)
226	NM_006622	SNK (serum-inducible kinase)
227	NM_001274	CHEK1 (CHK1 checkpoint homolog)
228	NM_003957 AJ006701 AF020089	STK29; PEN11B
229	NM_013233	STK39; SPAK
230	NM_003691	STK16; PKL12
231	XM_290796 AY049015 AK024376	TAO1/KIAA1361
232	NM_003159	STK9
233	NM_014586	HUNK (hormonally upregulated Neu-associated kinase)
234	NM_004834 NM_145686 NM_145687	MAP4K4; NIK; HGK
235	NM_002953	RPS6KA1 = ribosomal protein S6 kinase, 90kD, polypeptide 1
236	NM_021135	RPS6KA2 (ribosomal protein S6 kinase, 90kD, polypeptide 2); RSK3
237	NM_003161	RPS6KB1 (ribosomal protein S6 kinase, 70kD, polypeptide 1)

No.	Accession Number	Gene
238	NM_004586	RPS6KA3 = ribosomal protein S6 kinase, 90kD, polypeptide 3; RSK2
239	NM_004755	RPS6KA5 (ribosomal protein S6 kinase, 90kD, polypeptide 5); MSK1
240	NM_003942	RPS6KA4 (ribosomal protein S6 kinase, 90kD, polypeptide 4); MSK2
241	NM_003952	RPS6KB2 (ribosomal protein S6 kinase, 70kD, polypeptide 2)
242	NM_004760	STK17A; DRAK1
243	NM_014413	HRI (heme-regulated initiation factor 2-alpha kinase)
244	NM_007194	CHEK2 (CHK2 checkpoint homolog)
245	NM_012119	CCRK (cell cycle related kinase)
246	NM_014370	STK23; MSSK1
247	NM_005990	STK10; LOK
248	NM_004836	EIF2AK3 (eukaryotic translation initiation factor 2-alpha kinase 3)
249	NM_003618	MAP4K3; GLK
250	NM_014720	SLK (SNF1 sucrose nonfermenting like kinase)
251	NM_014602	PIK3R4 (phosphoinositide-3-kinase, regulatory subunit 4, p150)
252	NM_006285	TESK1 (testis-specific kinase 1)
253	NM_021643	GS3955 protein
254	NM_004203	PKMYT1

No.	Accession Number	Gene
255	NM_015148	PASK (PAS domain containing serine/threonine kinase)
256	NM_014002	IKKE (IKK-related kinase epsilon; inducible IkappaB kinase)
257	NM_007118	TRIO (triple functional domain (PTPRF interacting))
258	NM_001396	DYRK1A (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A)
259	NM_004714 NM_006483 NM_006484	DYRK1B (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1B)
260	NM_003583 NM_006482	DYRK2 (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2)
261	NM_003582	DYRK3 (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3)
262	NM_003845 AX166542 (longer at N-Term, C-Term different)	DYRK4 (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4)
263	NM_031417	MARKL1 (MAP/microtubule affinity-regulating kinase like 1)
264	NM_014840	KIAA0537 gene product
265	XM_039796 8 splice variants AF172264 - AF172271	TNIK (Traf2 and NCK interacting kinase)
266	XM_038150 AB011133	MAST3, KIAA0561 protein
267	XM_291141 AX166512 cds: 1-7572 AX766336 cds: 347-7636	MAST4, KIAA0303 protein
268	NM_015375 AB007941	DustyPK
269	NM_002760	PRKY (protein kinase, Y-linked)

No.	Accession Number	Gene
270	NM_003688	CASK (calcium/calmodulin-dependent serine protein kinase (MAGUK family))
271	NM_004734	DCAMKL1 (doublecortin and CaM kinase-like 1)
272	NM_152619 AX056380 cds: 7 - 2082	hypothetical protein MGC45428, DCAMKL2
273	AX504237 XM_047355 XM_047355 with ATG with ATG	DCAMKL3, KIAA1765 protein
274	NM_004226	STK17B; DRAK2
275	NM_005813	PRKCN (protein kinase C, nu)
276	NM_005255	GAK (cyclin G associated kinase)
277	NM_032294	hypothetical protein DKFZp761M0423
278	NM_014226	RAGE1 (renal tumor antigen)
279	NM_006035	CDC42BPB (CDC42 binding protein kinase beta (DMPK-like))
280	NM_007170	TESK2 (testis-specific kinase 2)
281	NM_152696	Nbak2, KIAA0630 protein
282	NM_016151	PSK
283	NM_173354 AX224729 cds: 112-2463	SNF1LK, SIK
284	AB023190	SAST (syntrophin associated serine/threonine kinase)
285	NM_022740	HIPK2 (homeodomain interacting protein kinase 2)
286	AX236110 cds:63-5012 AB037759	GCN2, eIF2alpha kinase
287	NM_013355	PKNbeta
288	NM_198465 AX504249 cds: 294-5039	NRK/ZC4 (NIK-related kinase)
289	NM_013257	SGKL (serum/glucocorticoid regulated kinase-like)

No.	Accession Number	Gene
290	NM_016276	SGK2 (serum/glucocorticoid regulated kinase 2)
291	NM_012424	RPS6KC1 (ribosomal protein S6 kinase, 52kD, polypeptide 1)
292	NM_014496	RPS6KA6 (ribosomal protein S6 kinase, 90kD, polypeptide 6); RSK4
293	NM_013254	TBK1 (TANK-binding kinase 1)
294	NM_016281	JIK
295	NM_016440	VRK3 for vaccinia related kinase 3
296	NM_015716	MINK (Misshapen/NIK-related kinase)
297	AX166520 cds: 1-1032	similar to Ca2+/Calmodulin-dependent protein kinase I, CAMK1b
298	NM_006410	HTATIP2 (HIV-1 Tat interactive protein 2, 30 kD)
299	NM_016542	MST4
300	NM_016653	ZAK (sterile-alpha motif and leucine zipper containing kinase AZK)
301	NM_173575	PKE, YANK3
302	NM_018979	PRKWINK1 (protein kinase, lysine deficient 1); WNK1
303	NM_006648 AX540385	PRKWINK2 (protein kinase, lysine deficient 2)
304	NM_020922 AJ409088 AX207405 splice variant?	PRKWINK3 (protein kinase, lysine deficient 3)
305	NM_032387	PRKWINK4 (protein kinase, lysine deficient 4)
306	NM_018492	TOPK (T-LAK cell-originated protein kinase)
307	AL359916 NM_080836 (longer at 5')	STK35, CLIK1

No.	Accession Number	Gene
308	NM_020680	NTKL (N-terminal kinase-like)
309	NM_032844	MASTL, hypothetical protein FLJ14813
310	NM_020397 NM_153498	CKLiK, CamKI-like protein kinase
311	AX224725 cds:1-2379 AB037781 NM_017988 (longer 3')	SCYL2
312	NM_153335 AF308302 AF308302	STLK5, LYK5
313	NM_174944 AX056447 cds: 372-1247	TSSK4
314	NM_052841	STK22C; TSSK3
315	XM_166453 AB058758	TTBK1
316	AR004796 U43586 XM_290793	KSR1 (kinase suppressor of ras)
317	NM_032037	SSTK
318	NM_016457	PKD2 (polycystic kidney disease 2)
319	NM_025195	C8FW, Trb1
320	NM_033266	ERN2 (ER to nucleus signalling 2)
321	NM_020423	PACE-1
322	NM_033550 AB017505	PRPK
323	NM_018401	serine/threonine kinase HSA250839, YANK2
324	NM_020639	ANKRD3 (ankyrin repeat domain 3); DIK
325	NM_015690	STK36
326	NM_014572	LATS2 (LATS, large tumor suppressor, homolog 2)
327	AX056397 cds:7-6861 AB037718	SPEG, KIAA1297 protein
328	AX504253 cds: 1-1704	Wee1B
329	AX766335 cds:1-4110 AB023216 NM_025164 shorter	QSK, KIAA0999 protein

No.	Accession Number	Gene
330	NM_007064	TRAD
331	NM_004690	LATS1 (LATS, large tumor suppressor, homolog 1)
332	NM_014911	AAK1
333	NM_014920 NM_016513 NM_173041	ICK, MAK-related kinase
334	NM_198892 NM_017593	BMP2K, BIKE
335	NM_033126	PSKH2
336	NM_031464	hypothetical protein MGC11287 similar to ribosomal protein S6 kinase
337	NM_032409	PINK1 (PTEN induced putative protein kinase 1)
338	NM_013392	NRBP (nuclear receptor binding protein
339	NM_016507	CrkRS
340	NM_005109	OSR1 (oxidative-stress responsive 1)
341	NM_139158	ALS2CR7
342	NM_032028 AY028964	STK22D, TSSK1
343	NM_017771	PXK (PX domain-containing protein kinase), Slob
344	NM_018571	ALS2CR2 (amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 2), STLK6
345	NM_031965	GSG2, haspin
346	NM_015191	SIK2, QIK
347	AX039412 cds:60 - 7850	KIAA1639, Obscn
		N-Term missing

No.	Accession Number	Gene
348	AX207388 cds: 404 - 1591 NM_145001	YANK1
349	AX394712 cds:282-1448 XM_373109	similar to MLCK, hypothetical protein LOC340156
350	NM_178510 AX207411 cds: 54 - 2348	ANKK1
351	NM_021158	C20orf97 (chromosome 20 open reading frame 97), Trb3
352	NM_152649 AX224735 cds: 465 - 1880	MLKL, hypothetical protein FLJ34389
353	AX250159 cds: 1 - 3735 XM_291277	Sgk223, DKFZp761P0423
354	XM_370878 AX250160	KIAA2002
355	NM_024652 AX250161 cds: 1 - 6044	LRRK1
356	NM_033115 AX250162 cds: 358-3039	TBCK, hypothetical portein MGC16169
357	AX250163 cds: 1-1682	Sgk424, similar to testis expressed gene 14 (LOC126392)
358	NM_031272 NM_198393 AX250165 cds: 123 - 4409	TEX14 (testis expressed sequence 14)
359	NM_024046	hypothetical protein MGC8407, VACAMKL
360	NM_014916	LMTK2, KIAA1079 protein, LMR2, KPI-2
361	NM_017433	MYO3A
362	NM_138995	MYO3B
363	NM_030952	SNARK
364	NM_030906 AJ303380	STK33

No.	Accession Number	Gene
365	NM_182493	similar to myosin light chain kinase (MLCK)
366	NM_032430 AB058714	BRSK1, KIAA1811
367	XM_370948 AX166553 cds: 1 - 1275	SBK, similar to SH3-binding kinase (LOC388228)
368	NM_032017	SINK-homologous serine/threonine kinase, MGC4796
369	NM_020547	AMHR2 (anti-Mullerian hormone receptor, type II)
370	NM_031414 NM_032944	STK31
371	NM_032237	hypothetical protein FLJ23356
372	NM_021133	RNASEL (ribonuclease L (2',5'-oligoadenylate synthetase-dependent))
373	AX166516 XM_372749	similar to protein kinase Bsk146
374	NM_153361	NIM1, MGC42105, similar to serine/threonine kinase (KIN1/SNF1/Nim1 subfamily)
375	NM_145203	casein kinase 1 alpha S-like, CK1a2
376	NM_173500 AX262516 cds: 317 - 4051	TTBK2
377	NM_144685	HIPK4
378	NM_175866 NM_144624 AX262519	KIS
379	AX166547 cds: 1 - 2835 NM_173598	KSR2
380	AX056416 cds: 28 - AR448352 cds: cds:282- AL137662 1529	NRBP2
381	AX540378 cds: 192-1541	SgK494, hypothetical protein FLJ25006
382	NM_152835	CLIK1L

No.	Accession Number	Gene
383	AX540373 cds: 195-2073 XM_376950	SgK071, similar to MGC43306 protein (LOC401568)
384	AX056460 cds: 1 - 1623 XM_038576	SgK493, hypothetical protein BC007901 (LOC91461)
385	NM_005157 NM_007313	ABL1
386	NM_005158 NM_007314	ABL2, ARG
387	NM_005781	ACK1
388	NM_000061	BTK
389	NM_005246	FER
390	NM_002005	FES
391	NM_002031	FRK (fyn-related kinase)
392	NM_002037 NM_153047 NM_153048	FYN
393	NM_002110	HCK
394	NM_005248	FGR
395	NM_005356	LCK
396	NM_002344	LTK
397	NM_002350	LYN
398	NM_004383	CSK
399	NM_005546	ITK
400	NM_005417 NM_198291	SRC
401	NM_003215	TEC
402	NM_005433	YES
403	NM_003328	TXK
404	NM_080823 AL121829 genomic clone	SRMS

No.	Accession Number	Gene
405	NM_001715	BLK
406	NM_001721	BMX
407	NM_005975	PTK6
408	NM_002821	PTK7
409	NM_002822	PTK9
410	NM_007284	PTK9L
411	NM_000222	KIT
412	NM_005211	CSF1R
413	NM_005232	EphA1
414	NM_004431	EphA2
415	NM_005233 NM_182644	EphA3
416	NM_004438	EphA4
417	NM_004439 NM_182472	EphA5
418	AX250164 cds: 280- XM_114973 3390	EphA6
419	NM_004440	EphA7
420	NM_020526	EphA8
421	AX166562 cds: 74 - 3100	EphA10
422	NM_004441	EphB1
423	NM_004442 NM_017449 AF025304	EphB2
424	NM_004443	EphB3
425	NM_004444	EphB4
426	NM_004445	EphB6

No.	Accession Number	Gene
427	NM_000604 9 transcripts NM_023109	FGFR1
428	NM_000141 13 transcripts NM_023028	FGFR2
429	NM_000142 NM_022965	FGFR3
430	NM_002011 NM_022963	FGFR4
431	NM_002253	KDR
432	NM_002019	FLT1
433	NM_004119	FLT3
434	NM_002020	FLT4
435	NM_005228	EGFR
436	NM_004448	HER2
437	NM_001982	HER3
438	NM_005235	HER4
439	NM_002378	MATK
440	NM_000875	IGF1R
441	NM_000208	INSR
442	NM_014215	INSRR
443	NM_002227	JAK1
444	NM_004972	JAK2
445	NM_000215	JAK3
446	NM_003331	TYK2
447	NM_006343	MER
448	NM_021913 NM_001699	AXL

No.	Accession Number	Gene
449	NM_006293	TYRO3
450	NM_000245	MET
451	NM_002447	MST1R, RON
452	NM_002958	RYK
453	NM_006206	PDGFRalpha
454	NM_002609	PDGFRbeta
455	NM_020630 NM_020629 NM_000323 NM_020975	RET
456	NM_005012	ROR1
457	NM_004560	ROR2
458	NM_002944	ROS1
459	NM_005607 NM_153831	PTK2, FAK
460	NM_004103 NM_173174 NM_173175 NM_173176	PTK2B, PYK2
461	NM_003177	SYK
462	NM_001079	ZAP70
463	NM_005424	TIE1
464	NM_000459	TEK, TIE2
465	NM_005592	MUSK
466	NM_002529	NTRK1
467	NM_006180	NTRK2
468	NM_002530	NTRK3
469	NM_013994 NM_001954 NM_013993	DDR1
470	NM_006182	DDR2
471	NM_004920	AATK/LMR1

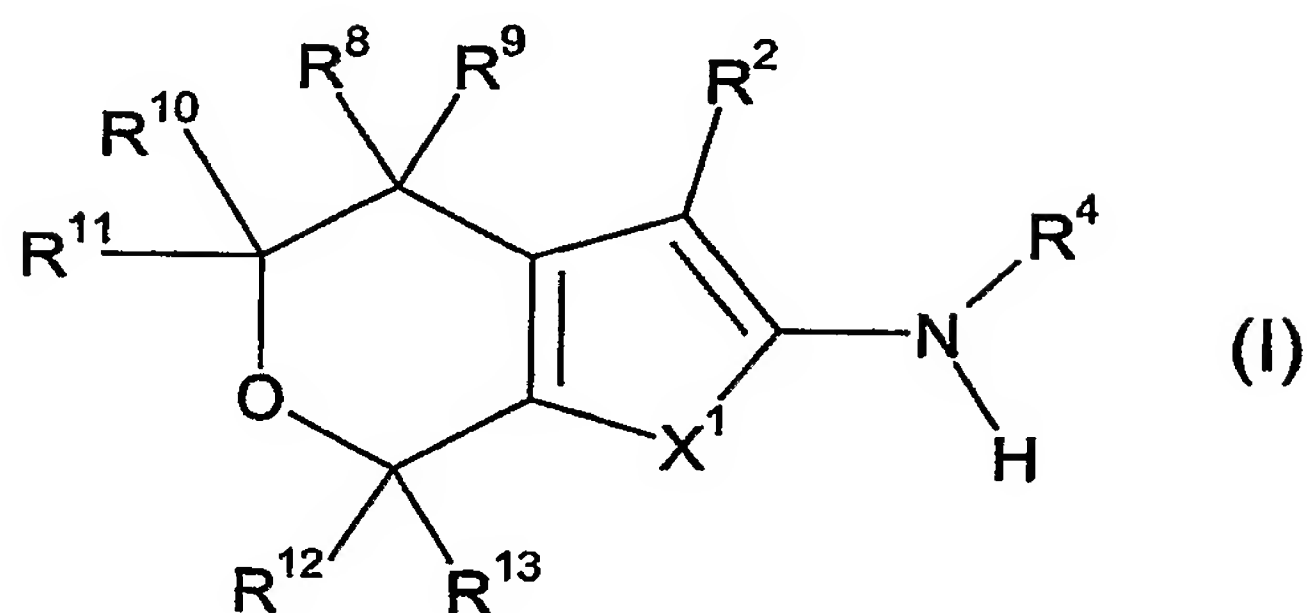
No.	Accession Number	Gene
472	XM_055866	LMTK3
473	NM_003985	TNK1
474	L08961	HUMSPRMTK
475	NM_004304	ALK
476	NM_015978	CARK
477	NM_018423	DKFZp761P1010
478	NM_032435 AJ311798	KIAA1804, MLK4
479	AJ277481	ILK-2
480	NM_000906	NPR1
481	NM_000907 NM_003995	NPR2
482	NM_004963	GUCY2C
483	NM_000180	GUCY2D
484	NM_001522	GUCY2F
485	XM_058513 AX166563	DKFZp434H2111
486		
487	NM_006218	PIK3CA (phosphoinositide-3-kinase, catalytic, alpha polypeptide)
488	NM_006219	PIK3CB (phosphoinositide-3-kinase, catalytic, beta polypeptide)
489	NM_002649	PIK3CG (phosphoinositide-3-kinase, catalytic, gamma polypeptide)
490	NM_005026	PIK3CD (phosphoinositide-3-kinase, catalytic, delta polypeptide)

No.	Accession Number	Gene
491	NM_014006	SMG1
492	NM_000051	ATM (ataxia telangiectasia mutated)
493	NM_001184	ATR (ataxia telangiectasia and Rad3 related)
494	NM_014216	ITPK1
495	NM_004958	FRAP1 (FK506 binding protein 12-rapamycin associated protein 1)
496	NM_002645	PIK3C2A (phosphoinositide-3-kinase, class 2, alpha polypeptide)
497	NM_002647	PIK3C3 (phosphoinositide-3-kinase, class 3); Vps34
498	NM_002651	PIK4CB (phosphatidylinositol 4-kinase, catalytic, beta polypeptide)
499	NM_002650	PIK4CA (phosphatidylinositol 4-kinase, catalytic, alpha polypeptide)
500	NM_003496	TRRAP (transformation/transcription domain-associated protein)
501	NM_002646	PIK3C2B (phosphoinositide-3-kinase, class 2, beta polypeptide)
502	NM_004570	PIK3C2G (phosphoinositide-3-kinase, class 2, gamma polypeptide)
503	NM_006904	PRKDC (protein kinase, DNA-activated)
504	NM_013302	elongation factor-2 kinase
505	NM_025144	LAK (lymphocyte alpha-kinase)
506	NM_017662	TRPM6

No.	Accession Number	Gene
507	NM_052947	HAK
508	NM_020778	MIDORI
509	NM_005881	BCKDK
510	NM_002610	PDK1
511	NM_002611	PDK2
512	NM_005391	PDK3
513	NM_002612	PDK4
514	NM_018343	RIOK2
515	NM_031480	RIOK1
516	NM_003831	RIOK3
517	BC017459	ADCK1
518	NM_052853	ADCK2
519	NM_020247	CABC1
520	NM_024876	ADCK4
521	NM_174922	ADCK5
522	NM_032454	STK19
523	NM_001726	BRDT
524	NM_005104	BRD2
525	NM_007371	BRD3
526	NM_058243	BRD4, var. long
527	NM_014299	BRD4, var. Short
528	NM_004606	TAF1
529	NM_153809	TAF1L

No.	Accession Number	Gene
530	NM_003852	TIF1
531	NM_005762	TRIM28
532	NM_015906	TRIM33

1. Compounds having the general formula (I)

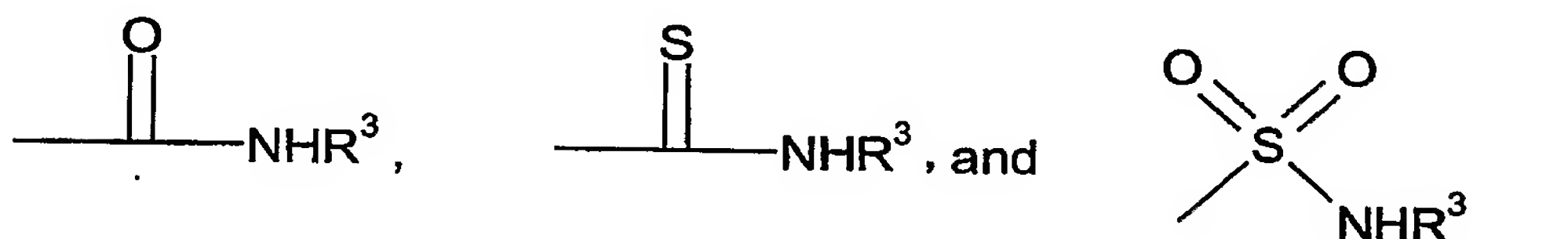


wherein

X^1 is selected from S, O, NR^1 ,

and R^1 is selected from H, substituted or unsubstituted C_1 - C_6 -alkyl,

R^2 is selected from



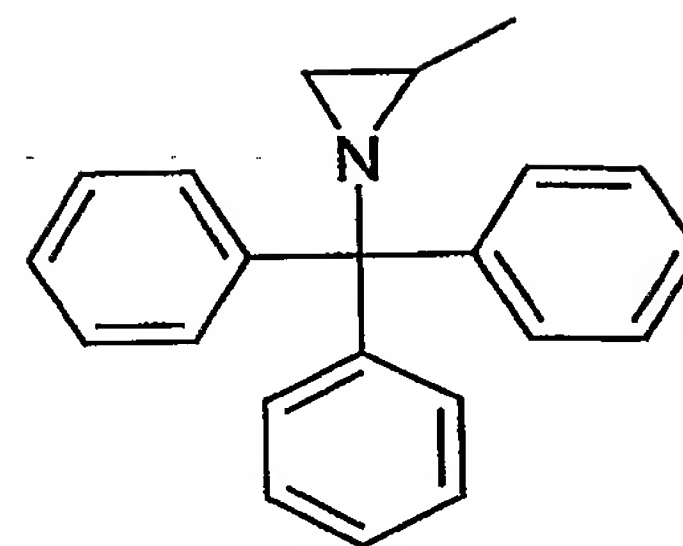
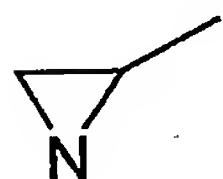
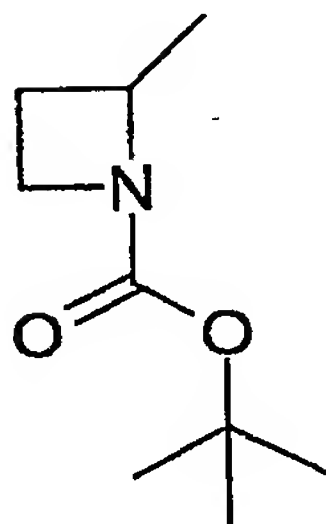
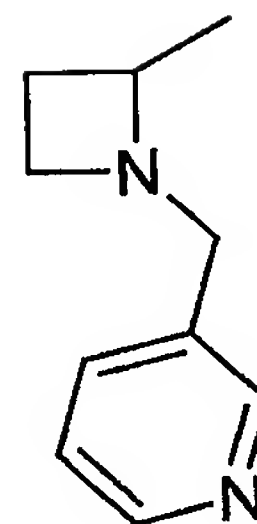
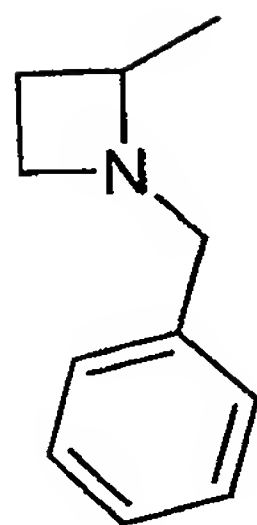
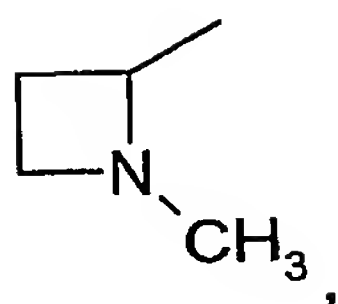
wherein R^3 is selected from H, HO-substituted, H_2N -substituted or HS-substituted C_1 - C_6 -alkyl,

R^4 is selected from H, $-C(=X^2)R^5$ and $-SO_2R^5$,

wherein X^2 is O, S or NH and

R^5 is selected from substituted or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, aryl, heteroaryl, heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, adamantyl,





or $-(\text{CH}_2)_n\text{-NR}^{14}\text{R}^{15}$,

wherein R^{14} and R^{15} are independently selected from substituted or
 5 unsubstituted $\text{C}_1\text{-C}_4\text{-alkyl}$ or $\text{C}_2\text{-C}_4\text{-alkenyl}$ and wherein $n = 1$ to 6,
 or NR^6R^7 ,

wherein

R^6 is selected from H, $\text{C}_1\text{-C}_6\text{-alkyl}$, and

R^7 is selected from substituted or unsubstituted $\text{C}_3\text{-C}_6\text{-cycloalkyl}$, $\text{C}_1\text{-C}_6\text{-alkyl}$,
 10 aryl, heteroaryl, heterocycloalkyl, $\text{C}_2\text{-C}_4\text{-alkenyl}$, $\text{C}_2\text{-C}_4\text{-alkinyl}$, or adamantyl,

R^8 is H and R^9 is selected from H, substituted or unsubstituted $\text{C}_1\text{-C}_6\text{-alkyl}$

R^{10} is selected from H, substituted or unsubstituted $\text{C}_1\text{-C}_6\text{-alkyl}$, $\text{C}_1\text{-C}_6\text{-alkoxy}$,
 or OH

15 R_{11} is selected from H and substituted or unsubstituted $\text{C}_1\text{-C}_6\text{-alkyl}$

R_{12} is selected from H and substituted or unsubstituted $\text{C}_1\text{-C}_6\text{-alkyl}$, $\text{C}_1\text{-C}_6\text{-alkoxy}$, or OH, and

R^{13} is selected from H or substituted or unsubstituted $\text{C}_1\text{-C}_6\text{-alkyl}$,

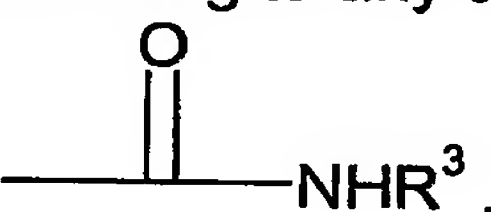
20 and stereoisomeric and regioisomeric forms and pharmaceutically acceptable
 salts of these compounds.

2. The compound according to claim 1, wherein X^1 is S.

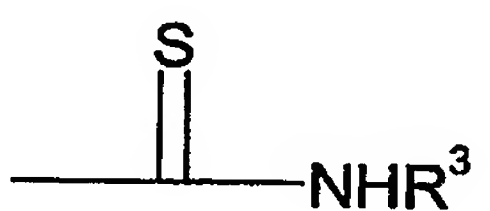
3. The compound according to claim 1, wherein X^1 is NR^1 , and R^1 is selected from H, substituted or unsubstituted C_1 - C_6 -alkyl, and preferably is methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl, or benzyl.

4. The compound according to claim 1, wherein X^1 is O.

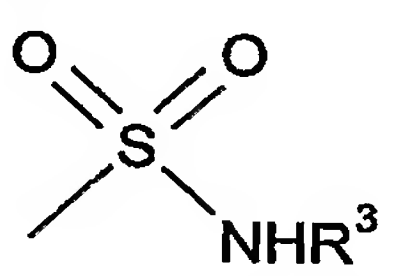
5. The compound according to any one of claims 1 to 4,

15 wherein R^2 is ,
and R^3 is selected from H, HO-substituted, H_2N -substituted or HS-substituted C_1 - C_4 -alkyl, and preferably is H.

6. The compound according to any one of claims 1 to 4,

25 wherein R^2 is ,
and R^3 is selected from H, HO-substituted, H_2N -substituted or HS-substituted C_1 - C_4 -alkyl, and preferably is H.

7. The compound according to any one of claims 1 to 4,

30 wherein R^2 is ,
and R^3 is selected from H, HO-substituted, H_2N -substituted or HS-substituted C_1 - C_4 -alkyl, and preferably is H.

35 8. The compound according to any one of claims 1 to 7,
wherein R^3 is selected from the group consisting of H, $-CH_2-CH_2-OH$, $-CH_2-CH_2-NH_2$, $-CH_2-CH_2-SH$, $-CH_2-CH(OH)-CH_3$, $-CH_2-CH(SH)-CH_3$, or $-CH_2-CH(NH_2)-CH_3$.

9. The compound according to any one of claims 1 to 8,
wherein R^4 is $-C(=X^2)R^5$ and X^2 is O or S.

10. The compound according to claim 9, wherein X^2 is O.

11. The compound according to any one of the preceding claims, wherein $R^4 = SO_2-R^5$.

12. The compound according to any one of claims 1 to 11,
wherein R^5 is selected from the group consisting of substituted or
unsubstituted methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl,
tert.-butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1 - C_6 cycloalkyles
substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl,
pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, *cis*-prop-1-
enyl, *trans*-prop-1-enyl, *cis*-prop-2-enyl, *trans*-prop-2-enyl, but-1-enyl, *cis*-but-
2-enyl, *trans*-but-2-enyl, but-3-enyl, prop-1-ynyl, prop-2-ynyl, but-1-ynyl, but-2-
ynyl, but-3-ynyl, adamantyl, or NR^6R^7 , wherein R^6 is H and R^7 is selected from
substituted or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, aryl, heteroaryl,
heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, or adamantyl.

13. The compound according to any one of claims 1 to 12,
wherein R^5 is selected from the group consisting of cyclopropyl, cyclobutyl,
cyclopentyl, cyclohexyl, phenyl-substituted cyclopropyl, cyclobutyl, cyclopentyl,
or cyclohexyl, methyl-substituted cyclopropyl, cyclobutyl, cyclopentyl, or
cyclohexyl, carboxyl substituted cyclopropyl, cyclobutyl, cyclopentyl, or
cyclohexyl, furanyl, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-
butyl, tert.-butyl, *cis*- or *trans*-prop-1-enyl, but-1-enyl, adamantyl, 3,4-
difluorophenyl or NR^6R^7 , wherein R^6 is H and R^7 is selected from substituted
or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, aryl, heteroaryl,
heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, or adamantyl, and R^7 preferably
is unsubstituted cyclohexyl, phenyl, 3,4-difluorophenyl, 4-acetylphenyl, p-
tolyl-phenyl or 4-fluorophenyl.

14. The compound according to claim 13, wherein R^7 is selected from substituted or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, heteroaryl, heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, or adamantyl.
- 5 15. The compound according to claim 13 or 14, wherein R^7 is selected from substituted or unsubstituted C_3 - C_6 -cycloalkyl, C_1 - C_6 -alkyl, aryl, heteroaryl, heterocycloalkyl, C_2 - C_4 -alkenyl, C_2 - C_4 -alkinyl, or adamantyl, and R^{10} is selected from H, substituted or unsubstituted C_1 - C_6 -alkoxy, or OH.
- 10 16. The compound according to any one of claims 1 to 15, wherein R^8 is H and R^9 is selected from H, or substituted or unsubstituted C_1 - C_6 -alkyl.
- 15 17. The compound according to any one of claims 1 to 16, wherein R^8 and R^9 are both H.
- 20 18. The compound according to any one of claims 1 to 17, wherein R^{10} , R^{11} , R^{12} , and R^{13} are independently selected from H and substituted or unsubstituted C_1 - C_6 -alkyl, and preferably from H or methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl or tert.-butyl.
- 25 19. The compound according to any one of claims 1 to 17, wherein R^{10} and R^{11} are methyl and R^{12} and R^{13} are H or wherein R^{10} , R^{11} , R^{12} , and R^{13} are H or wherein R^{10} , R^{11} , R^{12} , and R^{13} are methyl or R^{10} and R^{11} are H and R^{12} and R^{13} are methyl.
- 30 20. The compound according to any one of claims 1 to 17, wherein R^{10} is selected from substituted or unsubstituted C_1 - C_6 -alkoxy or OH and R^{11} is selected from H or substituted or unsubstituted C_1 - C_6 -alkyl.
21. The compound according to any one of claims 1 to 17, wherein R^{12} is selected from substituted or unsubstituted C_1 - C_6 -alkoxy or OH and R^{13} is selected from H or substituted or unsubstituted C_1 - C_6 -alkyl.

22. The compound according to any one of claims 1 to 21, wherein R^1 is selected from the group consisting of methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl or benzyl.

5 23. The compound according to any one of claims 1 to 22, wherein R^{14} and R^{15} are independently selected from methyl, ethyl and n-propyl, iso-propyl or allyl, and preferably are methyl.

10 24. The compound according to anyone of claims 1 to 23, wherein the compound is selected from the group consisting of:
 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 1),
 2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 2),
 15 2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 3),
 2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 4),
 20 2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 5),
 2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 6),
 2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 7),
 25 2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 8),
 2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 9),
 2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 10),
 30 2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 11),
 2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 12),

- 2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 13),
- 2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 14),
- 5 2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 15),
- 5,5-Dimethyl-2-(3-phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 16),
- 10 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-sulfonamide (Compound 17),
- 2-(3-Cyclohexyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 18),
- 2-(3-Phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 19),
- 15 2-[3-(4-Acetyl-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 20),
- 2-(3-p-Tolyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 21), and
- 20 2-[3-(4-Fluoro-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 22).
25. A compound according to claims 1 to 24 for use as a pharmaceutically active agent.
- 25 26. Use of at least one compound according to one of claims 1 to 24 as a pharmaceutically active agent.
27. Use of at least one compound according to one of claims 1 to 24 for the preparation of a medicament for the treatment of infectious diseases, including opportunistic diseases, particularly bacterially and/or virally induced infectious diseases, including opportunistic diseases.
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28. Use according to claim 25 or 26, for the prophylaxis and/or treatment of infectious diseases, including opportunistic diseases, particularly bacterially and/or virally induced infectious diseases, including opportunistic diseases.
- 5 29. Use according to claim 27 or 28, wherein the bacterially induced infectious disease is one caused by a bacterium of the genus *legionella*.
30. Use according to claim 29, wherein the disease is legionnaires' disease.
- 10 31. Use according to claim 27 or 28, wherein the bacterially induced infectious disease is caused by a mycobacterium.
32. Use according to claim 31, wherein the mycobacterium is *Mycobacterium tuberculosis* or *Mycobacterium leprae*.
- 15 33. Use according to claim 31 or 32, wherein the infectious disease is tuberculosis, leprosy or mycobacterially induced meningitis.
34. Use according to claim 27 or 28, wherein the virally induced infectious disease is one caused by a hepadnavirus.
- 20 35. Use according to claim 33, wherein the hepadnavirus is selected from HBV, GSHV or WHV.
- 25 36. Use of at least one compound according to one of claims 1 to 24 for the preparation of a medicament for the treatment of autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke.
- 30 37. Use of at least one compound according to one of claims 1 to 24 for the prophylaxis and/or treatment of autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke.

38. Use according to one of claims 36 or 37, wherein the autoimmune diseases are selected from the group comprising: asthma, chronic obstructive pulmonary diseases, systemic lupus erythematosus, immune mediated glomerulonephritis, rheumatoid arthritis, osteoporosis, psoriasis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, alopecia or autoimmune diabetes mellitus.
39. Use according to one of claims 36 or 37, wherein the cardiovascular diseases are selected from the group consisting of: Adult congenital heart disease, aneurysms, angina, angina pectoris, arrhythmias, cardiovascular disease prevention, cardiomyopathies, congestive heart failure, myocardial infarction, pulmonary hypertension, hypertrophic growth, restenosis, stenosis or arteriosclerosis.
40. Use according to one of claims 36 or 37, wherein the cell proliferative disease is cancer.
41. Use according to claim 40, wherein the cancer is selected from the group consisting of: Bladder, breast, central nervous system, colon, gastric, lung, kidney, melanoma, head and neck, ovarian, cervix, glioblastoma, pancreas, prostate, stomach, skin, testis, leukaemia, Hodgkin's lymphoma, liver and renal cancer.
42. Use according to one of claims 36 or 37, wherein said diabetes is selected from Type I diabetes or Type II diabetes.
43. Use according to one of claims 36 or 37, wherein said inflammation is mediated by cytokines, such as TNF- α , IL-1 β , GM-CSF, IL-6 and/or IL-8.
44. Use according to one of claims 36 or 37, wherein the neurodegenerative diseases are selected from the group comprising: Alzheimer's disease, Parkinson's disease, AIDS-related dementia, Huntington's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, spinal muscular atrophy and cerebellar degeneration.

45. Use of a compound according to any one of claims 1 to 24 as an inhibitor for a protein kinase.
- 5 46. Use according to claim 43, wherein the protein kinase is a mycobacterial kinase.
47. Use according to claim 46, wherein the protein kinase is from *Mycobacterium tuberculosis* or *Mycobacterium leprae*.
- 10 48. Use according to claim 47, wherein the protein kinase from *Mycobacterium tuberculosis* or *Mycobacterium leprae* is protein kinase G (PknG).
49. Use according to claim 45, wherein the protein kinase is a cellular kinase.
- 15 50. Use according to claim 49, wherein the protein kinase is selected from the group consisting of: EGFR, PDGF, c-kit, c-Src, GSK-3, CDK1 or SRPK1.
- 51 Use of at least one compound according to claims 1 to 24 for the preparation
20 of a pharmaceutical composition.
52. Use according to claim 51, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of bacterially and/or virally induced infectious diseases, including opportunistic diseases.
- 25 53. Use according to claim 52, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of bacterially induced infectious diseases caused by a bacterium of the genus legionella.
- 30 54. Use according to claim 52, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of legionnaires' disease.

55. Use according to claim 52, wherein the bacterially induced infectious disease is caused by a mycobacterium, preferably *Mycobacterium tuberculosis* or *Mycobacterium leprae*.
- 5 56. Use according to claim 54 or 55, wherein the infectious disease is tuberculosis, leprosy or mycobacterially induced meningitis.
- 10 57. Use according to claim 52, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of virally induced infectious diseases, caused by a hepadnavirus.
58. Use according to claim 57, wherein the hepadnavirus is selected from HBV, GSHV or WHV.
- 15 59. Use according to claim 52, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke.
- 20 60. Use according to claim 59, wherein the autoimmune diseases are selected from the group comprising: asthma, chronic obstructive pulmonary diseases, systemic lupus erythematosus, immune mediated glomerulonephritis, rheumatoid arthritis, osteoporosis, psoriasis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, alopecia or autoimmune diabetes mellitus.
- 25 61. Use according to claim 59, wherein the cardiovascular diseases are selected from the group consisting of: Adult congenital heart disease, aneurysms, angina, angina pectoris, arrhythmias, cardiovascular disease prevention, cardiomyopathies, congestive heart failure, myocardial infarction, pulmonary hypertension, hypertrophic growth, restenosis, stenosis or arteriosclerosis.
- 30

62. Use according to claim 59, wherein the cell proliferative disease is cancer, wherein the cancer is selected from the group comprising:
Bladder, breast, central nervous system, colon, gastric, lung, kidney, melanoma, head and neck, ovarian, cervix, glioblastoma, pancreas, prostate, stomach, skin testis, leukaemia, Hodgkin's lymphoma, liver and renal cancer.
63. Use according to claim 59, wherein said diabetes is selected from Type I diabetes or Type II diabetes.
64. Use according to claim 59, wherein said inflammation is mediated by cytokines, such as TNF- α , IL-1 β , GM-CSF, IL-6 and/or IL-8.
65. Use according to claim 59, wherein the neurodegenerative diseases are selected from the group comprising: Alzheimer's disease, Parkinson's disease, AIDS-related dementia, Huntington's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, spinal muscular atrophy and cerebellar degeneration.
66. Use according to any one of claims 51 to 65, wherein the compound inhibits a protein kinase.
67. Use according to claim 66, wherein the protein kinase is a mycobacterial kinase.
68. Use according to claim 67, wherein the protein kinase is from *Mycobacterium tuberculosis* or *Mycobacterium leprae*.
69. Use according to claim 68, wherein the protein kinase from *Mycobacterium tuberculosis* or *Mycobacterium leprae* is protein kinase G (PknG).
70. Use according to claim 66, wherein the protein kinase is a cellular kinase.
71. Use according to claim 70, wherein the protein kinase is selected from the group consisting of: EGFR, PDGF, c-kit, c-Src, GSK-3, CDK1 or SRPK1.

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Summary

5 Described are 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and their analogues and
pharmaceutically acceptable salts thereof, the use of these derivatives for the
prophylaxis and/or treatment of mycobacteria-induced infections, opportunistic
infections, autoimmune diseases, bipolar disorders, cardiovascular diseases, cell
proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and
stroke, as well as compositions containing at least one 7-dihydro-5H-
10 thieno[2,3c]pyran derivatives and their analogues derivative and/or pharmaceutically
acceptable salts thereof.

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